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1. Goldenberg, Marcel; Apgar, Virginia; Deterling, Ralph; and Pines, K. L.: *J. A. M. A.*, 140:776, July 2, 1949.

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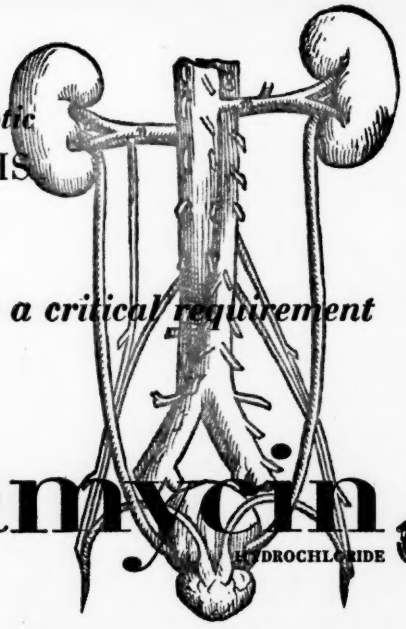

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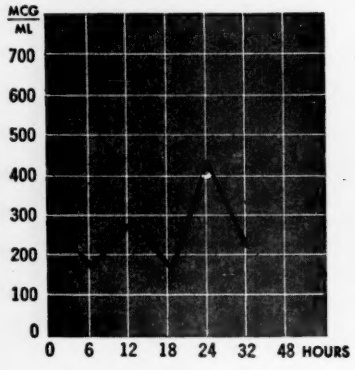

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THE ADRENAL-HYALURONIDASE RELATIONSHIP IN INFECTION*

GEO. H. SMITH AND JEANETTE C. OPSAHL†

A few of us can still recall the shocked expression that filled the faces of our instructors when Charles V. Chapin, in his curt and incisive terms, reported the somewhat conflicting views of his more ponderous predecessor, George M. Sternberg, to the effect that a definite and fixed host-parasite relationship does not always exist. Chapin expressed belief that while there are a few cases of host-parasite relationships that clearly show a definite phase outcome, each has its infinite variety of form and manifestations, and each may vary in accord with its own capacities and capabilities—that for every outcome which is predictable, there are many that do not lend themselves to such clearly defined expression; hence, variations in the parasite within the host in each individual case determine what may develop.

Although this concept, which led to the acceptance of bacterial diseases as well as of carriers, greatly disturbed some of our elders, it formed a notable turning point in our knowledge of bacteriology—a knowledge which is even now being intensified by a study of some of the factors involved.

That the problem of infection and resistance is becoming more complex each year is clearly seen when we consider recent studies of glandular derivatives and antibiotics. The terms "resistance to infection" and "susceptibility to infection" were adopted by bacteriologists, as well as by many others, who had no clear concept of what the meaning of these terms should be; and since these terms had no exact meaning, they were used freely and often without regard to their fitness. Thus it was that at the turn of the century it became apparent that vagueness of ideas was often expressed without the necessity of precision, and the same is true today.

The present study involves the effects of derivatives of the adrenal cortex on the spreading of actual parasites in the host. It is hoped that this research will permit us to penetrate to some degree into currents and cross currents that are operative in many different diseases.

The spreading throughout tissues of particulate matter, such as the carbon in India ink, can be enhanced by the action of hyaluronidase. Many bacteria also spread from a localized focus, and it has been shown that this spreading may be facilitated by the presence of hyaluronidase or some similar agent.[2] Since both adrenal cortical hormones and hyaluronidase are said to play important rôles in processes of infection, it seems reasonable to suppose that the two systems might interact in some way.

The literature on the subjects of bacterial infection and resistance is practically inexhaustible, and since the discovery of spreading factors by Duran-Reynals,[1] almost innumerable investigations as to their rôles in infectious processes have been reported.[3, 5, 7] The relationship of the adrenals to infection is discussed in part in a review of adrenal cortical hypertrophy

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by Tepperman, Engel, and Long.[16] They note that the adrenals of animals with acute and chronic infections are frequently enlarged, and they state that "the obvious lack of specificity of this reaction immediately suggests that it is not the infection itself which produces the adrenal hypertrophy, but rather some metabolic change that occurs as a result of the infection."

Many individual studies have been made of the effects of cortical extracts or of adrenalectomy relative to isolated disease pathology, but results for the most part are inconclusive except for the well-known evidence that adrenalectomy causes a definite decrease in resistance to infection.[4, 6, 12–14] Scott, Bradford, Hartman, and McCoy[15] tried to ascertain whether the injection of adrenal extracts would increase the resistance of animals with intact adrenals against intoxications or infection; however, no benefit was shown, and whether the doses used (adequate to maintain adrenalectomized animals) were insufficient for the tremendous load of the intoxication or infection, or whether, in the animals tested, an adrenal cortical deficiency played no important part in the outcome was not determined. Vollmer[17] reported the use of whole adrenal cortical extracts in experimental infections and concluded that ". . . despite occasional evidence that adrenal cortical extracts administered during infection may benefit intact animals, a clear understanding of the rôle of the adrenal cortex in resistance to disease may require further extensive exploration."

The purpose of this investigation was to relate the infectious activity of two bacterial species, *Staphylococcus aureus* and *Salmonella aertrycke,* to the rôle of the adrenal cortex in resistance to infection.

Experimental

Since the presence of hyaluronidase in various strains of staphylococci has been shown by several investigators,[2] a spreading factor was assumed to be present in the staphylococcus strain used in this study. When preliminary experiments showed that broth culture medium also contained a factor that seemed to enhance the spreading of India ink, a confirmatory study was made using the same *Staphylococcus aureus* grown in a beef heart infusion culture medium, which was diluted with varying amounts of saline, mixed with India ink, and injected intradermally into mice of the inbred CBA strain. The general methods have been described in some detail in previously published reports from this laboratory.[8, 9, 11]

Evidence that the broth culture medium enhanced the spread of India ink is presented in Table 1. On diluting the culture medium with saline, the spreading effect was decreased. The fact that a filtrate of the bacterial culture gave much larger areas of spread than did the culture medium alone, and also that dilution with saline had relatively less effect than in the case of the bacterial culture, substantiates the assumption that the strain of *Staphylococcus aureus* used contained an active spreading factor, most likely hyaluronidase.

This strain of *Staphylococcus aureus* was grown for 16 hours in a culture medium of beef heart infusion, and 0.1 cc. was injected intradermally into mice, 10 to 12 weeks of age. This inoculum did not cause death in normal animals, but after one or two days severe lesions did occur at the site of injection. The severity of the infection was graded from 0 to 4 plus. Since, in the previous experiments the broth culture medium alone caused an enhancement of the spread of India ink, it was considered necessary to repeat the infection studies by injecting intradermally 0.1 cc. of a saline suspension of the bacterial organisms. For this purpose, the bacteria were grown for 16 hours on agar slants containing a beef heart infusion, and then 10 cc. of normal saline were used to remove the bacterial colonies. Of this suspension, 0.1 cc. was used as the infectious agent. In Table 2 results are shown for the injection of either the broth culture or the saline suspen-

TABLE 1

DEMONSTRATION OF SPREADING FACTOR IN 16-HOUR BROTH CULTURES OF STAPHYLOCOCCUS AUREUS

Dilution of bacterial filtrate or broth culture medium	*Area of spread*	
	Bacterial filtrate	*Broth culture medium*
Undiluted 1:1	514 sq. mm.	293 sq. mm.
Diluted 1:2	450	220
Diluted 1:5	400	162
Diluted 1:10	418	127

Area of spread in mice three hours after the intradermal injection of 0.1 cc. of India ink combined with a diluted filtrate of *Staphylococcus aureus* culture in broth or combined with diluted broth alone (eight mice in each group).

sion of *Staphylococcus aureus* into normal mice, normal mice pretreated with adrenal cortical extract (Wilson's), adrenalectomized mice, and into adrenalectomized mice that had been pretreated with adrenal cortical extract.

As noted above, the inoculum employed was not lethal for normal animals, but very severe lesions (4 plus) resulted in from two to four days following infection. The normal animals given 2 cc. of aqueous adrenal cortical extract intraperitoneally three hours prior to the administration of the infectious agent had a marked resistance to the development of lesions. The adrenalectomized mice appeared to develop severe lesions more rapidly than did the normal animals, but the most outstanding observation was the high mortality as early as one day after infection. With both types of inoculum, all of the infected adrenalectomized animals died within seven days. Replacement therapy with adrenal cortical extract in the adrenalectomized animals increased their resistance to the infection, as evidenced by a

TABLE 2

INFLUENCE OF ADRENALECTOMY AND A.C.E. ON THE COURSE OF INFECTION RESULTING FROM THE INTRADERMAL INJECTION OF STAPHYLOCOCCUS AUREUS INTO 12-WEEK-OLD MICE OF THE INBRED CBA STRAIN

Inoculum	*Experimental group*	*Time of observation—days**						
		1	2	3	4	5	6	7
0.1 cc. of 16-hour broth culture	Normal (12)	++ (0/12)	+++ (0/12)	+++ (0/12)	++++ (0/12)	++ (0/12)	++ (0/12)	± (0/12)
	Normal (6) A.C.E., 2 cc. I.P. 3 hours before infection	± (0/6)	± (0/6)	+ (0/6)	+ (0/6)	± (0/6)	± (0/6)	0 (0/6)
	Adrex (12) 24 hours before infection	++ (7/12)	++++ (8/12)	++++ (8/12)	++++ (10/12)	++++ (10/12)	++++ (10/12)	(12/12)
0.1 cc. saline suspension of 16-hour beef heart infusion agar slant	Normal (6)	++ (0/6)	++++ (0/6)	++++ (0/6)	++++ (0/6)	++ (0/6)	++ (0/6)	± (0/6)
	Normal (6) A.C.E., 2 cc. I.P. 3 hours before infection	± (0/6)	+ (0/6)	± (0/6)	± (0/6)	± (0/6)	0	0
	Adrex (7) 6 days before infection	++ (2/7)	+++ (3/7)	++++ (4/7)	+++ (5/7)	++++ (5/7)	+++ (5/7)	(7/7)
	Adrex (7) 6 days before infection. A.C.E., 2 cc. I.P. 3 hours before infection	± (0/7)	+ (1/7)	++ (1/7)	++ (2/7)	+++ (2/7)	+++ (2/7)	++ (3/7)

* The fractions denote the number of dead over total number used in the experiment.

lowered mortality and by the development of less severe lesions. It is obvious, however, that replacement therapy was not complete, due either to inadequate amounts or improper timing of extract administration.

Since protection following the injection of adrenal cortical extract was observed in these experiments, it was considered likely that a part of this protection could have been due to an inhibition of the bacterial spreading factor by the adrenal cortical steroids of the extract. In order to investigate further this possibility, a suspension of *Staphylococcus aureus* in saline was mixed with an equal amount of adrenal cortical extract, and this mixture

TABLE 3

SIMULTANEOUS INOCULATION OF A SUSPENSION OF STAPHYLOCOCCUS AUREUS IN SALINE, AND A.C.E. (OR MERTHIOLATE ALONE) IN NORMAL AND ADRENALECTOMIZED MICE*

Inoculum	*Time of observation, days*				
	1	2	3	6	9
		Normal mice			
0.05 cc. bacterial suspension (B.S.) plus 0.05 cc. saline	±	++	+++	+++	±
0.05 cc. B.S. plus 0.05 cc. Merthiolate (1 : 100,000)	±	+	++	+++	±
0.05 cc. B.S. plus 0.05 cc. A.C.E.	0	±	+	0	0
		Adrenalectomized mice			
0.05 cc. B.S. plus 0.05 cc. saline	±	++	++++	++++	++++
0.05 cc. B.S. plus 0.05 cc. Merthiolate (1 : 100,000)	±	+	++	++++	++++ (3 dead)
0.05 cc. B.S. plus 0.05 cc. A.C.E.	±	+	+	+	0

* Six mice in each group.

was injected intradermally into normal and into adrenalectomized mice. The adrenal cortical extract used in these studies contained Merthiolate 1 : 100,000, thus making it necessary to control the exeriments by giving a mixture of Merthiolate and the bacterial suspension. A third control group was employed in which saline was mixed with the bacterial suspension. The results of some of these studies are presented in Table 3. It is apparent that the small amount of Merthiolate present in the adrenal cortical extract was not sufficient to afford any protection. However, the adrenal cortical extract provided marked protection, as evidenced by the

development of smaller and less severe lesions than those in the control groups. The protective effect of adrenal cortical extract was seen in both normal and in adrenalectomized animals. It should be noted that the dosage of adrenal cortical extract used in these experiments was considerably smaller than that used in the previously cited groups of experiments. The results obtained parallel very closely the inhibition of the spreading phenomenon by adrenal cortical steroids, as reported earlier.[8, 10, 11]

The investigations of Duran-Reynals and others have suggested that *Salmonella aertrycke* does not contain the enzyme hyaluronidase. Inasmuch as this organism is extremely infectious for mice, it was chosen for the second series of studies. *Salmonella aertrycke* was grown for 16 hours on agar slants containing beef heart infusion. A suspension of the bacterial colonies was made in saline and injected intradermally into mice. A preliminary study showed that either 0.1 or 0.05 cc. of the suspension would kill all of the normal control animals. Therefore 0.05 cc. was chosen as the arbitrary dosage for the experimental studies. This amount of the bacterial suspension was injected intradermally into normal and into adrenalectomized mice, and adrenal cortical therapy was carried out by the administration of either aqueous adrenal cortical extract (Wilson's) or Lipo-adrenal extract (Upjohn's). When the aqueous extract was used, 1.0 cc. was given three hours before the injection of the bacterial suspension, and 1.0 cc. was injected each day for eight days following infection. The Lipo-adrenal extract was administered in a dosage of 0.2 cc., 48 hours and again six hours before injection of the bacterial suspension. On the second day after infection, an additional dose of 0.2 cc. was administered.

The extreme toxicity of this organism for mice is evident from the data given in Table 4; within a period of ten days all infected animals died. The administration of adrenal cortical extract, either aqueous or Lipo-adrenal extract, did not appear to influence in any major degree the course of infection in the normal animals. However, it is obvious that the adrenalectomized animals were to some degree protected by adrenal cortical therapy. As was the case in the *Staphylococcus aureus* studies, replacement therapy was not complete, since the adrenalectomized animals receiving adrenal cortical extract did not survive as long as did the controls.

Discussion

It has been well established that the spreading factor is an important agent governing invasion of bacteria through the barrier provided by the ground substance of the mesenchyme.[1, 2] The results of the present investigation support this conclusion in an indirect manner. The inhibitory effect of adrenal cortical extracts and certain adrenal cortical steroids on hyaluronidase-enhanced spreading of India ink has been demonstrated by previous studies.[8, 10, 11] If the invasiveness of bacteria is affected by the presence of the enzyme hyaluronidase, the invasion of *Staphylococcus*

aureus should be enhanced in the adrenalectomized animals and suppressed by the administration of adrenal cortical extract. On the other hand, *Salmonella aertrycke,* which presumably does not contain the enzyme hyaluronidase, should not be affected in the same manner by the absence of

TABLE 4

INFLUENCE OF ADRENALECTOMY AND A.C.E. (AQUEOUS) OR LIPO-ADRENAL EXTRACT ON THE COURSE OF INFECTION RESULTING FROM THE INTRADERMAL INJECTION OF 0.05 CC. OF A SUSPENSION OF S. AERTRYCKE GROWN FOR 16 HOURS ON BEEF BROTH AGAR SLANTS

	Time of observation, days				
Experimental group	1	2	3	6	10
Normal controls	± (0/6)*	++ (0/6)	+++ (0/6)	++++ (3/6)	(6/6)
Normal with A.C.E. (aqueous) 1 cc. prior to infection and 1 cc. each day for 8 days	0 (0/6)	± (0/6)	+ (0/6)	++++ (5/6)	(6/6)
Adrex	± (5/6)	(6/6)			
Adrex with A.C.E. (aqueous) 1 cc. prior to infection and 1 cc. each day for 8 days	0 (1/6)	++ (3/6)	+++ (3/6)	(6/6)	
Normal controls	+ (0/6)	++ (0/6)	+++ (0/6)	++++ (4/6)	(6/6)
Normal with Lipo-extract 0.2 cc. 48 hours before and 0.2 cc. 6 hours before infection. 0.2 cc. on the 2nd day after infection.	+ (0/6)	+ (0/6)	++ (0/6)	++++ (5/6)	(6/6)
Adrex	± (4/6)	(6/6)			
Adrex with Lipo-extract 0.2 cc. 48 hours and 6 hours before infection. 0.2 cc. on the 2nd day following infection.	± (1/6)	+ (1/6)	(6/6)		

* Denotes the number of mice dead over total number used in this experiment.

the adrenals or by the administration of adrenal extracts. The extreme sensitivity of the adrenalectomized animal to all types of infection, as well as to many types of non-specific stress, makes it apparent that the relationship between the adrenal hormones and the hyaluronidase can be only one

of several factors involved. Therefore, it can not be expected that all of the effects of the adrenal cortex on resistance to infection will be related to hyaluronidase, although this may be one factor of importance.

Contrary to the protection afforded by adrenal cortical extracts in infections with *Staphylococcus aureus,* little or no effect was observed on the course of infection in normal animals injected with *Salmonella aertrycke.* The experiments with this organism were not so extensive nor so clear-cut as were the staphylococcus studies; however, it seems to be fairly well established that the adrenal cortical extracts did not provide any measurable protection in the normal animal. Replacement therapy in the adrenalectomized animal resulted in a definite protection, although its demonstration was only on the basis of prolonging life. The adrenalectomized animals given either aqueous or Lipo-adrenal extract did not survive for as long as did the normal controls. The amount of adrenal extract required for complete replacement therapy is known to be great,[5] and the doses employed in this study were known to be suboptimal, but larger doses probably would have been impractical. It is possible that by increasing the amount of adrenal extract administered or of the effective fractions thereof, some protection of the normal animals might have been obtained, since the adrenal steroids offer resistance to stress in ways not involving the adrenal-hyaluronidase relationship.

The experiments with *Staphylococcus aureus* show that adrenal cortical extracts have a pronounced inhibitory effect on the development of lesions. This was noted when adrenal cortical extracts were administered to normal or to adrenalectomized animals. The most conclusive evidence that the adrenal hormones exerted part of their effect through an inhibition of bacterial hyaluronidase is derived from the experiments in which adrenal cortical extracts and bacterial suspensions were mixed together and administered simultaneously. Under these conditions a definite protection was observed. The dosage of adrenal cortical extract was minute in comparison to the amount that would be required for replacement therapy. It is difficult to draw conclusions other than that the protection was due to an inhibition of hyaluronidase present in the bacterial suspension.

Summary

1. The influence of adrenalectomy and adrenal cortical extracts on the course of infection resulting from the intradermal injection of a hyaluronidase-containing bacterium, *Staphylococcus aureus,* and of a hyaluronidase-free bacterium, *Salmonella aertrycke,* has been investigated.

2. With the hyaluronidase-containing bacteria, the course of infection could be inhibited markedly in normal animals as well as in adrenalectomized animals by the administration of adrenal cortical extracts.

3. When adrenal cortical extract was mixed with the bacterial suspension and the mixture injected intradermally in mice, the course of infection was inhibited. Under these conditions the amount of adrenal cortical extract necessary for protection was minute when compared with the amount required for protection when administered systemically.

4. In the case of the hyaluronidase-free bacteria, a protective effect of adrenal cortical extract could be demonstrated only in the adrenalectomized animal. With both types of organism, replacement therapy with adrenal cortical extract in the adrenalectomized animal did not give as complete a protection against infection as was found in the normal control animals.

5. It has been concluded that the adrenal-hyaluronidase relationship may be one of the factors involved in the lowered resistance of adrenalectomized animals to infection, but a lack of hyaluronidase inhibition can be only one component of this complicated deficiency condition.

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THE AILMENTS AND PHYSICIANS OF DR. JOHNSON*

PETER PINEO CHASE

Boswell and other writers have made Johnson probably the most intimately known man of all time. His medical history is almost as explicit. With the exception of the period from early manhood to middle age—in general, a time lacking in medical incidents—we can follow his case history from before birth through his autopsy two days after death.

Johnson's mother was forty years old when she had her first labor; an "elderly primip," as we said at the Boston Lying-In. Mr. Hector, the man midwife, reported that she had a long, difficult labor, that the child was almost dead and was unable to cry for some time.[1] As Joseph Priestley, the Unitarian minister whom Johnson abhorred, had not as yet isolated oxygen, naturally it was not then appreciated that the child was presumably extremely anoxic. We know now that such a birth condition may show its effects on the brain long after. Later in the paper this will be discussed.

Within a few weeks the baby had an abscess of the buttock, which might well have been due to a burn, as was then suspected. He was soon put to a wet nurse who had already suckled her child for eighteen months. Possibly this was harder on the nurse than on the child. This woman's son had scrofula, ominous of Samuel's exhibition of the same trouble later.[2] Presumably, the bovine tuberculosis can be transmitted by human as well as cow's milk, and in a few years young Johnson had tuberculous glands of the neck treated by the royal touch of Queen Anne.[3] In the early years of my practice we saw many cases of this disease—in fact, at the hospital we had a special clinic devoted to its care. As I look back on our experiences I have no conviction that our therapeusis was better than that of the Stuart lady.

Johnson's poor sight in his left eye, his deafness in his left ear, and the blemishes on the skin of his face have always been considered the aftermath of his scrofulous infection. I wonder if all of this was true. Tuberculous and syphilitic keratitis were rather common in those days. Since we have not the slightest reason to suspect lues in the respectable Johnson family, there is a perfectly good chance, but no certainty, that his eye trouble was tuberculous.

We may have much more skepticism about his deafness having the same etiology. Tuberculosis of the middle ear is a chronic destructive disease, in many cases involving the mastoid bone and nearly always perforating the

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* Presented before the Beaumont Medical Club on December 8, 1950.

[1] Reade, A. L.: *Johnsonian gleanings.* Part X, p. 20. Privately printed, 1946.

[2] *Ibid.*, p. 22.

[3] *Ibid.*, p. 25.

ear drum with a long-drawn-out discharge, disagreeable and malodorous. Mention is not made of such a condition in Johnson, and it would certainly have attracted attention.

There are numerous references in Boswell to Johnson's poor complexion. Aleyn Reade says: "The scrofula too . . . had left its marks deeply graven on his face." I have consulted some dermatologists about this point. Tuberculous lupus was formerly rather common but it was decidedly characteristic, especially during the stage of its active process, which is not described in Johnson's case. One dermatologist told me that it was uncommon to have facial skin lesions associated with scrofula.[4] Reade tells us that little Samuel at some unrecorded date had the smallpox; in the early eighteenth century he would have been exceptional had he escaped it. Smallpox sometimes affected the eyes, it was common for it to cause deafness, and its facial disfigurement was notorious. I think it is highly probable that it played a large part, with bovine tuberculosis, in producing Johnson's physical defects.[5] There is no evidence suggesting that human tuberculosis, that which attacks the lungs, ever affected Johnson's health.

He thus had a difficult childhood and he grew up into a tall, bony young man, deaf, half blind, disfigured by scars, given to queer gesticulations and mutterings, and moody and often unsociable. Is it any wonder that he was a failure as a teacher of youth?

Henry James in his life of Charles W. Eliot points out that Eliot's hemangioma, covering the whole right side of his face, caused him to be aloof and to make little attempt to ingratiate himself with others or even to adopt the ordinary ways of intercourse. It strongly influenced his career, allowing him to proceed undeviatingly along the way he had mapped out, unembarrassed by the compromises which handicap the rest of us. It is probable that Johnson's difficulties were the deciding factor in making his career as great at it was. A man of such unusual faculties as he possessed might well have been a great educator had he had a good start. Nevertheless, it is hard to believe that his pre-eminent capacity as a scholar, author, and philosopher would have asserted itself in the routine of school life. He was free to follow his own inclination all his life and despite his own, not altogether true, line, his worth was not "by poverty depressed."

After his arrival in London, his disfigured features, his physical disabilities, his mannerisms, and his unsociable and forbidding ways served to free him from the many distractions that familiar association with the gay world provides. Pretty young streetwalkers would not bestow their favors on him as they did on the charming Boswell and he was too poor to buy them. Of course none of the more polite circles were open to such an uncouth character until he had proved his worth. So he consorted with

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[4] Personal communications from dermatologists.

[5] Handbook of the common acute infectious diseases. Auralgan Research Division, 1949.

Savage, some of the booksellers and publishers, who thought him a useful hack, and with a few authors. Thus, he had freedom to develop his knowledge and talents.

For many years his physical health was evidently good. He was powerful, able to pick up a man and chair and throw them off the stage; he rode to hounds. When he was sixty-four years old he traveled through Scotland and the Hebrides with Boswell, mostly on horseback and in small boats through wicked, tide-swept waters. How many Yale graduates of sixty-four would feel up to a trip through Patagonia and by sailboat through the Strait of Magellan? I imagine this would be much less than what Johnson did. Yet he had not been absolutely free of symptoms. There are numerous references to flatulence in his letters, which I would take to mean that he had symptoms of indigestion. This is not remarkable when you consider his table habits. He told Mrs. Thrale: "My genius is always in extremes." He could be abstinent but when he let himself go with food he raised a "gustatory perspiration."

Much has been made of his tea drinking habit and the number of cups he consumed. But Percy Laithwaite, Secretary of the Johnson Society at Lichfield, called my attention to the minute size of the cups in Johnson's tea set, and a dozen or so of those was not a great amount of tea. Since his autopsy showed his stomach to be normal (and presumably his duodenum) but that he had a large gallstone, we now know the cause of his flatulence.

In 1780, when he was seventy-one years old he wrote Mrs. Thrale: "Last year I perceived the remission of those convulsions in my breast which had distressed me for more than twenty years." That takes him back several years previous to his meeting with Boswell. Through all these years, including the tour to the Hebrides, I find little in Boswell to suggest that Johnson was not in vigorous physical condition. Upon my telling this to a friend of mine, an able internist, he remarked: "Did you ever see B. in one of his fits of coughing? Would you not call that a convulsion in the breast?" Johnson probably had a chronic bronchitis. In fact, his autopsy bears this out.[8] "On opening the cavity of the chest, the lungs did not collapse as they usually do when air is admitted, but remained distended, as if they had lost the power of contraction; the air cells on the surface of the lungs were also very much enlarged." This is the picture of long standing emphysema produced by chronic bronchitis.

In the same seventies he said to Boswell: "It is now about the time when we were traveling (Hebrides) I have however better health than I had then." This and other letters show that until he was in the seventies he considered himself a strong, essentially well man.

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[8] Rolleston, Sir Humphry: Samuel Johnson's medical experiences. Ann. M. Hist., 1929, *n.s. 1*, 540. This contains the necropsy report on Johnson by James Wilson, F.R.S. The report is now in the Royal College of Physicians in London. It has been published in numerous papers.

This was not to last much longer. When he was seventy-two he wrote to Boswell: "My health has been tottering this last year." From then on he was troubled with shortness of breath and he frequently refers to being bled. This much-abused practice of the old days may well have been in Johnson's case good treatment, lowering his blood pressure. Mrs. Piozzi tells of Johnson, panting with asthma and dropsy, and his friend Dr. Lawrence, struck with a palsy, sitting among the latter's skeletons and monsters and writing Latin billets to each other. Johnson at this time took much opium for relief; he says three grains a day. He accentuates the fact that opium habitués are those who want to be such or those who would in any event be slaves to some bad habit. When he found that opium did not help him, he gave it up.

In his seventy-fourth year he had a paralytic stroke of which he himself gave a graphic account. He had aphasia and agraphia. "My speech was taken from me. I made a Latin prayer in verse. The lines were not good but I knew them not to be good. In penning a note I had some difficulty; my hand, I knew not how or why, made wrong letters." As this took place on Bunker Hill Day, which as all New Englanders know is June 17, and by the Fourth of July he had been to the Club and dined on fish, with the wing of a small turkey-chick, even though he left beef, goose, and venison pie untouched, and since he was then planning a trip into Kent the next week, it is safe to say he had not had a severe hemorrhage—probably a small thrombosis.

The relief from frequent blood lettings, plus the cerebral accident, certainly suggest that he had a high blood pressure. At autopsy his heart was found "exceedingly large and strong, the valves of the aorta were beginning to ossify." A cardiologist tells me this is consistent with high blood pressure but that his heart was not a good heart even though it appeared strong to his eighteenth century physicians; an enlarged heart is not a good one.

From now on he was a sick man. There are more and more references to his asthma, his dropsy and, to add to his tribulations, that most characteristic infirmity of the heavy eating, heavy drinking eighteenth century —gout. Later on in one of his remissions he says: "What a man am I who have got the better of three diseases—the palsy, the gout, and the asthma." It is evident that he was suffering from kidney disease, heart disease, or a combination, cardiorenal disease. His recurring attacks of what he refers to as asthma suggest a cardiac asthma. Possibly the fact that his dropsy was dependent points to the same conclusion.

That his kidneys were involved is shown by one episode. Ten months before his death, when he was evidently badly water-logged, he received "sudden and unexpected relief" by voiding twenty pints of urine in one day. Mercuric diuretics were not known at that time, but people then as now were obsessed by worry about their bowel function. Johnson was no

exception and we know he took "mercury," probably in the form of calomel, to stimulate these aforesaid viscera. As he had been extremely ill of asthma also, he probably had been forced to complete rest. Recovery from some degree of decompensation, plus the mercury, resulted in this tremendous diuresis.

There are also numerous references to a sarcocele. This is something that I have never run across in my modern medical studies. I presume it no longer exists except in the dictionary where it is defined as a fleshy tumor of the testicle. Johnson did not have a testicular new growth. He evidently had edema of the scrotum and a hydrocele which was drained. His surgeons apparently had considered at one time the removal of the testicle. We can understand his perturbation in pre-anesthetic days.

Nine months before his death his asthma and dropsy were bad enough to keep him confined for ten weeks. Boswell in desperation wrote to Cullen, Monro, and others of Edinburgh for advice.[7] Nevertheless, Johnson was soon attending the club and making rounds of calls. Six months before he died he visited Oxford—no mean jaunt over eighteenth century roads, although Trevelyan said they were rapidly improving. "Coaches had no springs, stoppages and overturns were frequent and the highwayman was still at the height of his glory." About this time Johnson was able to run up the entire length of the staircase of the Royal Academy.

We even find him visiting his home town of Lichfield a month before his death. Dr. Johnson evidently felt as Logan Clendening did—I quote from memory—"If you obey your doctors and do everything you ought to do, you will live a month longer; which month you will spend flat on your back instructing your nurse just whom you want for pallbearers." This home visit was his last fling. His asthma and dropsy became too much for him—the latter despite occasional scarifications by his surgeons and by himself on his last day of life. Undoubtedly, his mind was dull when he did this, but throughout his life he was interested in medicine and attempted intelligently to understand the reasoning back of diagnosis and treatment. He did not believe in the indiscriminate bleeding then common.

His judgmatical remarks regarding medical matters are scattered throughout Boswell. Thus, "Nay, sir, whatever may be the quantity that a man eats, it is plain that if he is too fat, he has eaten more than he should have done. One man may have a digestion that consumes food better than common; but it is a certainty that solidity is increased by putting something to it." A century and more later physicians and hospitals finally appreciated the wisdom contained in the following observation. "I have often thought that if I kept a seraglio the ladies should wear linen gowns or cotton—no silk—it will be very nasty before it is perceived to be so. Linen detects its own nastiness."

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[7] Letters from Drs. Cullen, Hope, and Monro. Boswell Papers, Yale University Library.

In one of his last days he insisted on using his own judgment as to his treatment. He asked Dr. Brocklesby if he could recover and when told that it would be a miracle if he did, he said: "Then, I will take no more physick, not even my opiates, for I have prayed that I may render up my soul to God unclouded." He little understood the blessed dullness that usually accompanies approaching death but he did pass an uncommon judgment on unnecessary medication.

Most of what the autopsy disclosed could have been deduced from a careful study of his case. The lungs were emphysematous; the heart was large but did not show valvular abnormalities. There was no fluid in the thorax or pericardium. The liver and spleen were hard with chronic passive congestion; a gallstone and evidences of inflammation in its vicinity explained his digestive condition. He had ascites; not unexpected with his history of swollen belly, scrotum, and legs.

The most interesting finding was the condition of his kidneys, which one would have suspected were diseased. "The kidney of the left side, some hydatids beginning to form on its surface; that of the right side was almost entirely destroyed, and two large hydatids formed in its place. Dr. Johnson never complained of any pain in this part; the left testicle had a number of hydatids on its surface, containing a fatty gelatinous fluid, the right testicle had hydatids also." The kidneys might have been expected to show nephritis. Possibly there was a hydronephrosis on the right but it does not sound like that—eighteenth century physicians knew pus when they saw it. Presumably there was a cystic degeneration of the kidneys—not polycystic kidneys, if this latter condition is an entity.

On the basis of a voluminous history, many recordings of physical findings, and an autopsy, I would hazard these diagnoses: Severe trauma and anoxia at childbirth; early infection with bovine tuberculosis, involving the cervical glands and possibly the eye; loss of function of the left ear, possibly due to birth trauma, possibly to smallpox; skin-scarring due to smallpox; cerebral irritation through life, the aftermath of birth injuries; chronic bronchitis, possibly some allergic asthma resulting in emphysema; hypertension due largely to cystic degeneration of the kidneys; cerebral thrombosis; hydrocele; his terminal condition, cardiorenal disease.

So much for his organic difficulties. What probably interests Dr. Johnson's acquaintances more are his psychic troubles. An English physician, W. Russell Brain, in discussing at length Johnson's case, says that Johnson's involuntary movements were not due to organic disease of the brain but were of psychological origin.[8,9] Katherine Balderston, in the book of essays presented to Professor Tinker, elaborates the modernistic view that Johnson's "deep-rooted psychic maladjustment" was founded on

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[8] Brain, W. Russell: Some reflections on genius. Lancet, 1948, *1*, 661-665.
[9] Brain, W. Russell: Authors and psychopaths. Brit. M. J., 1949, *2*, 1427.

"unrecognized erotic" ideas in his subconscious mind.[10] It would be decidedly amateurish for a mere surgeon to discuss the psychology of a middle-aged and elderly gentleman's sexual reactions. I have talked about this with several psychiatrists who evidently lay much emphasis on the sex background of mental aberrations. On the other hand, one of my consultants, older to be sure, is impatient with these ideas and feels that it is not necessary to go into the realm of erotic fantasy to explain these psychic maladjustments.[11]

Miss Balderston selects passages from Thraliana, telling of Johnson's strange, wild thoughts; these, as she says, are "scattered, dark, and difficult to interpret." Miss Balderston's theory is that Johnson had his "unrecognized erotic thoughts" about Mrs. Thrale and this would "explain the violence of his repudiation of Mrs. Thrale when she married Piozzi." But all his life Johnson had been violent in his reactions and this last episode occurred five months before his death, after he had had a stroke and was an exceedingly sick old man. His characteristic change of mood is also shown when six days later he wrote her: "I wish that God may grant you every blessing, . . . and whatever I can contribute to your happiness I am ever ready to repay." We must remember though that when anything popped into Mrs. Thrale's mind she popped immediately upstairs and popped it into her blank book. The rest of us have our fleeting thoughts about our associates, sometimes loving, sometimes the opposite, and often weird. They usually fade soon away. But thanks to Mrs. Thrale's energy, many difficult to classify little flies are embalmed in amber. We can clinch either of two sides of many an argument by Thraliana.

I am not as sure as is Dr. Brain that there was nothing organic in Dr. Johnson's nervous difficulties. For nearly a quarter of a century the Bradley Home in East Providence, Rhode Island has been studying maladjusted children—not feeble-minded children, indeed quite the opposite. Dr. Charles Bradley of the Bradley Home has found that children who have suffered prolonged anoxia at birth or in infancy often develop tics, mannerisms, and personality changes. They are distinctly subject to sudden changes of mood, like Dr. Johnson, who would verbally annihilate some unfortunate and immediately after treat him with great tenderness and consideration.[12-16] Far be it from me to deny Johnson subconscious erotic

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[10] Balderston, Katherine C.: Johnson's vile melancholy, pp. 3-14 in *The age of Johnson*. Essays presented to Chauncey Brewster Tinker. New Haven, Yale University Press, 1949.

[11] Shattuck, G. L.: Personal communication.

[12] Rosenfeld, G. B. and Bradley, Charles: Childhood behavior sequelae of asphyxia in infancy. Pediatrics, 1948, *2*, 74.

[13] Nelson, R. L.: The neurological complications of whooping cough. J. Pediat., S. Louis, 1939, *14*, 39.

[14] Darke, R. A.: Late effects of severe asphyxia neonatorum. J. Pediat., S. Louis, 1944, *24*, 148.

[15] Chornyak, J.: Some remarks on diagnosis of psychopathic delinquent. Am. J. Psych., 1941, *97*, 1326.

[16] Lurie, L. A. and Levy, Sol: Personality changes and behavior disorders of children following pertussis. J. Am. M. Ass., 1942, *120*, 891.

thoughts, but can we not reasonably explain him without these? A poor start at birth, scrofulous, half blind, deaf, disfigured features, unable to continue at Oxford, poor as a church mouse, a failure at teaching—he had reason to be melancholic. He tells of his despondency to Dr. Swynfen, who suggests that he might have a loss of reason in the future and makes public the young man's story.[17] Here is good etiology for melancholy and an abnormal mind. Herman Liebert, who is intimately acquainted with everything pertaining to Johnson, has also taken this attitude in his "Reflections on Dr. Johnson."[18]

The Superintendent of Butler Hospital,[19] having read Miss Balderston's essay, tells me she has much to support her contention. But I wonder if she has not put the cart before the horse. Dr. Johnson might have had queer subconscious erotic thoughts because of a queer mind; not vice versa. Dr. Wright reminds us not to forget the tremendous documentation of Johnson's life. Were we all so written up, erotic thoughts and all, would we have a clean mental bill of health?

Dr. Warbasse listed fifty-seven physicians associated with Samuel Johnson and he did not include them all.[20] Many of them were forced upon Johnson by his disabilities and many he voluntarily chose for friends, from Levett the quack who lived with him to Dr. Mead, the rich and famous, who Johnson said, "lived more in the broad sunshine of life than any man." Johnson's chief physician, the one who attended him throughout his last illness and was present at his autopsy, was William Heberden, the outstanding internist of the time and to this day a great name in medicine. He was the founder of the famous eponym, Heberden's nodes, much more familiar to me, a surgeon, than the actual little swellings themselves. Also the physician of John Hunter, it may have been that from observing Hunter he recognized and first described angina pectoris. It is just as well that Johnson and John Hunter were not associates, for the meeting of two such strong-minded and irascible men might well have precipitated Hunter's fatal angina.

Cruickshank was Johnson's surgeon but he had the advice and assistance of the versatile Percival Pott. Although William Hunter was a friend of Johnson they had no professional relations—Johnson requiring neither a gynecologist nor obstetrician. Many medical men gave their services to Johnson. The one who was closest to him in his later years was a delightful, rather John Bull type of man, Richard Brocklesby. All these physicians were fond of and admired Johnson and gave their services free. Brocklesby

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[17] Reade, A. L.: (note 1), p. 85.

[18] Liebert, H. W.: Reflections on Samuel Johnson. . . . J. English and German Philology, 1948, *37*, 80.

[19] Dr. David Wright.

[20] Warbasse, J. P.: Doctors of Samuel Johnson and his court. M. Lib. Hist. J., 1907, *5*, 65-87; 194-210; 260-272.

went further and offered him a pension which was, however, gratefully refused.

In the last pages of Boswell we read this—"About eight or ten days before his death, when Dr. Brocklesby paid him his morning visit, he seemed very low and desponding, and said, 'I have been as a dying man all night.' He then emphatically broke out in the words of Shakespeare:

> 'Cans't thou not minister to a mind diseas'd;
> Pluck from the memory a rooted sorrow;
> Raze out the written troubles of the brain;
> And with some sweet oblivious antidote,
> Cleanse the stuff'd bosom of that perilous stuff,
> Which weighs upon the heart?'

To which Dr. Brocklesby readily answer'd, from the same great poet: '. . . therein the patient must minister to himself.' Johnson expressed himself much satisfied with the application."

Two years ago at Haverford, Colonel Isham showed many of the new found Boswell papers. As Brocklesby is a hero to me, I asked if I might see a letter of his; this Colonel Isham graciously handed to me. The paper, finely preserved, was in striking contrast to our modern wood pulp stuff. The ink was unfaded and in Dr. Brocklesby's firm round hand, easily legible, was the story I have just quoted. But Boswell in the *Life* has cleverly edited it. Brocklesby had casually referred to the passage in Macbeth—Boswell, realizing that not all of us are minutely familiar with Shakespeare, had supplied the actual quotation.

Johnson was not acquainted with all the ablest medical men of his time in Great Britain. For example, Boswell writes thus of Sir John Pringle, the famous naval surgeon and hygienist.

> Sir John Pringle, "mine own friend and my father's friend," between whom and Dr. Johnson I in vain wished to establish an acquaintance, as I respected and lived in intimacy with both of them, observed to me once, very ingeniously, "It is not in friendship as in mathematics, where two things each equal to a third, are equal between themselves. You agree with Johnson as a middle quality, and you agree with me as a middle quality; but Johnson and I should not agree." Sir John was not sufficiently flexible; the repulsion was equally strong on the part of Johnson?

One very great medical man was a contemporary of Johnson and yet presumably unknown to him. The year after Johnson died William Withering brought out his account of the foxglove.[21] Had Withering had the opportunity to treat Johnson, he might possibly have prolonged his life in reasonable comfort. It is a commentary on the public estimate of importance that there is no article on Withering in my *Encyclopedia*

[21] Withering, William: *An account of the foxglove.* Birmingham, 1785.

Britannica, although a contemporary English actor named West Digges is given an inch or two.

These, then, are some random thoughts on the medical history of a great man who said: "I believe every man has found in physicians great liberality and dignity of sentiment, very prompt effusions of beneficence, and willingness to extend a lucrative art where there is no hope of lucre."

CIRCULATORY CHANGES IN CHRONIC PULMONARY DISEASE*

A Study of Pulmonary Collateral Circulation

FRANK D. GRAY, JR.,† PAUL R. LURIE,‡ and RUTH WHITTEMORE

With the demonstration by Bing and associates that extensive collateral circulation between systemic and pulmonary arteries develops in some types of cyanotic congenital heart disease,[4] the question of whether other clinical states associated with cyanosis might also exhibit collateral circulation presented itself. The answer has appeared in the remarkable injection studies of Liebow and associates, who clearly demonstrated anatomically the presence of large collateral vessels in lungs taken from cases of chronic pulmonary disease.[13] These vessels consist of precapillary anastomoses between bronchial arteries and pulmonary arteries.

The physiological significance, that is, the extent and consequences of such additional channels, remained to be demonstrated. Such demonstration, together with observations of other circulatory phenomena associated with chronic lung disease, constitute the purpose of the present report.

Methods

Material. Ten patients from the wards and clinic of the Grace-New Haven Community Hospital, in whom a diagnosis of some type of chronic pulmonary disease had been made, were the subjects of this report. The clinical findings are summarized in Table 1.

Technical procedures. After the clinical studies were completed, the patient was subjected to cardiac catheterization for the purposes of recording pressures in the heart and obtaining samples of mixed venous blood. During the period of the catheterization an arterial blood sample was obtained while the patient was breathing room air and again after breathing pure oxygen for five minutes.¶ Finally a sample of expired air was collected in a Douglas bag.

The blood gas analyses were done by the Ordway modification of the method of Roughton and Scholander.[18] The volume of the Douglas bag expired air sample was measured and then the sample was analyzed in the Scholander micro-gas analyzer.[19] The oxygen consumed by the patient in one minute was calculated according to a standard method.[8]

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† This work was done during the tenure of a James Hudson Brown Memorial Research Fellowship in Internal Medicine.

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¶ Five minutes of oxygen breathing will not produce a homogeneous oxygen milieu in the lungs of some victims of pulmonary disease. In our experience, however, very little change occurs after three minutes; hence five minutes was arbitrarily accepted as a safe and practical period.

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The patient was observed while in a resting, fasting state. The period of study began at 9:00 A.M. and usually ended sixty to ninety minutes later. No sedatives were used, but a great effort was made to allay the patient's anxiety about the procedure by the methods to be described in detail elsewhere.[15]

Calculations. The volume of blood flowing through systemic capillaries was calculated by means of the Fick principle[6, 7, 23]:

$$[1] \qquad \frac{O}{a - v} = SF$$

where O = Oxygen consumed per minute in cubic centimeters × 100.
a = Arterial blood oxygen content in volumes per cent.
v = Mixed venous blood oxygen content in volumes per cent.
SF = Systemic blood flow in cubic centimeters per minute.

Formulae for calculating the proportions of blood being shunted into the arterial system without oxygenation, as well as that being shunted from the aorta back into the pulmonary artery without passing through the systemic capillaries, were first demonstrated by Lundsgaard and Van Slyke.[14] They developed the formula for right-to-left shunts as follows:

$$[2] \qquad \frac{(T - LT) - a}{(T - LT) - v} = \alpha$$

where T = Total oxygen carrying capacity of hemoglobin in volumes per cent.
L = Fraction of hemoglobin passing through ventilated lung capillaries without becoming oxygenated.*
α = Net fraction of SF shunted from right-to-left; the net fraction is equal to the total mixed venous blood shunted from right-to-left less the mixed venous blood returned through left-to-right shunts.

(Lundsgaard and Van Slyke[14] used reduced hemoglobin values instead of oxyhemoglobin values. In this report their symbols have been changed to accord with present day usage.)

On referring to Figure 1 it is apparent that using (v) in equation [2] will result in a value for α representing, not the absolute right-to-left shunt of blood, but rather the algebraic sum of the right-to-left shunt of mixed venous blood diminished by the amount of mixed venous blood returned via collaterals. The total collateral (left-to-right) shunt expressed as a fraction of SF constitutes β in Figure 1.

$$\frac{(a_o - a_{air}) - (T_o - T_{air}) - (d_o - d_{air})}{T_{air}} = L$$

In which the symbols are used as in the text and the subscripts refer to observations during pure oxygen and room air breathing respectively.

In order to calculate the absolute shunt, one must substitute (v_1)* for (v) in equation 2, giving:

$$[3] \qquad \frac{(T - LT) - a}{(T - LT) - v_1} = \alpha$$

(v_1) (see Fig. 1) cannot be observed directly. If, however, the blood passing through β and mixing with (v) to form (v_1) could be rendered almost completely saturated, it would no longer influence the calculation, since the algebraic sum of the fractions of mixed venous blood in the right-to-left and left-to-right shunts would approach the

* L is calculated by Ordway's equation[16, 17] which is a mathematical expression of Berggren's work showing that breathing pure oxygen tends to saturate that proportion of blood going through functioning capillaries which, due to faulty ventilation or diffusion, is unsaturated while room air is breathed.[1] The equation states:

fraction representing the total right-to-left shunt. This can be effected by having the patient breathe pure oxygen. The resulting high alveolar oxygen tension places the arterial blood (therefore the blood going through the β shunt) on a part of the oxyhemoglobin dissociation curve where relatively large tension differences result in negligible changes in saturation.

Ordway has developed a modification of the Lundsgaard and Van Slyke formulae to use in cases when the subject is breathing pure oxygen[16, 17]:

$$[4] \quad \frac{(T + d - a)_o}{(T + d - a)_o + D} = a_o$$

where a, (T), and (a) are used as for equation [2] except that subscript ($_o$) means that they were estimated during pure oxygen breathing.

d = Oxygen physically dissolved in blood in volumes per cent. (d_o is considered to be 1.0 volume per cent in each case.[18])

D = a — v. (There is some variability of (a — v) when changing from breathing room air to breathing pure oxygen, but in this equation the variation is not significant.)

The value for a_o calculated in this manner therefore approaches the absolute right-to-left shunt. As long as (a) is not 100% saturated, the a_o so calculated will be less than the true absolute a (labelled a_1 in Fig. 1).

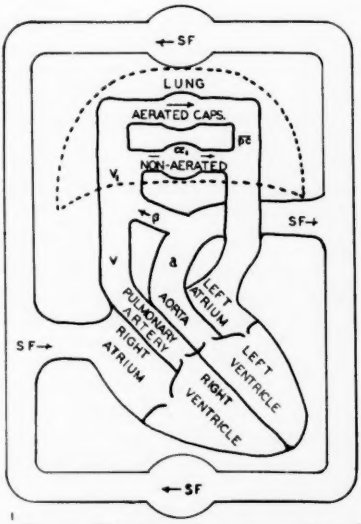

FIG. 1. Diagrammatic representation of the pulmonary circulation in chronic lung disease.

Legend. SF = Systemic blood flow; v = mixed venous blood oxygen content; v_1 = admixture of v with blood from collaterals, β; a = arterial blood oxygen content; a_1 = shunt through non-aerated pulmonary capillaries; β = shunt through collateral channels; pc = fully aerated blood oxygen content = (T + d — LT).

Knowing the approximate proportion of SF which is shunted through non-aerated lung (a_o), it is possible to calculate the approximate amount of the systemic flow (SF) going through aerated pulmonary capillaries, assuming that the mixed venous blood is distributed anatomically in the same proportions through aerated and non-aerated channels as is the total pulmonary blood supply, and that there is no intracardiac right-to-left shunt:

[5] SF $(1 - a_o)$ = aliquot of SF passing through aerated capillaries.

However, some mixed venous blood after passing through non-aerated pulmonary capillaries is returned to the pulmonary arteries via the β shunt to go through non-aerated, a_o, as well as aerated, $1 - a_o$, pulmonary capillaries. The total amount of mixed venous blood reaching aerated pulmonary capillaries by any route is the

* That (v_1) approaches homogeneity is suggested by the anatomical demonstrations of Liebow *et al.*[18]

effective pulmonary flow of Bing and associates.[5] This includes mixed venous blood originally shunted through non-aerated lung, then returned to aerated capillaries via bronchial to pulmonary artery collaterals:

$$[6] \qquad \frac{O}{(T - LT) - v} = EPF$$

where EPF is the effective pulmonary blood flow in cubic centimeters per minute.

If the EPF is greater than the proportion of SF going through aerated capillaries (equation [5]), then the difference must be due to mixed venous blood joining the pulmonary artery flow from the collateral circulation, β. This difference consists of mixed venous blood equal to the α_0 per cent of that part of β going to aerated capillaries. The part of β going to aerated capillaries is $(1 - \alpha_0)\ \beta$, and $\alpha_0\%$ of that may be written $\alpha_0(1 - \alpha_0)\beta$. This may be expressed in terms of volume flow by multiplying by SF, whereupon it becomes $\alpha_0(1 - \alpha_0)\beta$ SF. As stated above, this is equal to the difference between EPF and equation [5]; hence βSF may be estimated:

$$EPF - SF(1 - \alpha_0) = \alpha_0\ (1 - \alpha_0)\beta SF$$

or

$$[7] \qquad \frac{EPF - SF(1 - \alpha_0)}{\alpha_0(1 - \alpha_0)} = \beta SF.$$

If α_0 were exactly equal to a_1, equation [3] could be set equal to equation [4] and solved for (v_1). Then, using the Lundsgaard and Van Slyke equation for left-to-right shunts (β), the collateral flow could be calculated directly. The Lundsgaard and Van Slyke equation is as follows[14]:

$$[8] \qquad 1 - \frac{v_1 - a}{D} = \beta$$

The β calculated in this way is somewhat higher than that calculated by using equation [7] which was used throughout in the following report.

The accuracy of the direct Fick method of estimating systemic flow is well established.[6, 23] Since α_0 is the other factor entering prominently into the present calculation of βSF, an evaluation of the variation encountered in estimating α_0 was made. This was done by analysing the range of variation encountered in the laboratory determination of blood oxygen content. Each blood sample was analysed independently by two technicians; the average of the two determinations was accepted if they fell within 0.2 volumes per cent of one another. In this study the maximum range between two analyses on identical samples was ± 0.04 volume per cent for arterial blood samples and ± 0.08 volume per cent for venous blood samples. If these differences occurred in an additive direction, they could result in a maximum range of estimated value for α_0 of ± 2 (expressed as a per cent of SF). However, in many of the cases included here the maximum difference did not occur in every blood gas analysis, hence a range for each case was determined on the basis of individual laboratory data.

Assuming that the true value for blood oxygen content would fall somewhere between the results of the two independent analyses, the range of βSF calculated from these data is given in the last column of Table 3.

Results and discussion

The outstanding symptom of the patients in this study was dyspnea which was to some degree disabling in each case (Table 1). Frank left ventricular failure as indicated by paroxysmal dyspnea or signs of pulmonary congestion which cleared on digitalization was present in only

three cases. These three cases alone had peripheral edema; however, all cases except two (N.V. and H.H.) had distended neck veins. As may be seen from Table 2, the right ventricular or pulmonary arterial systolic pressure was elevated above 30 mm. Hg in four cases. In spite of cyanosis and clubbing in several cases, polycythemia was conspicuously rare in this group. In the small group reported here it is difficult to see any correlations between the clinical data and the observed physiological data.

All but three of these cases revealed a right-to-left shunt through inadequately aerated lung tissue. As may be seen in Tables 2 and 3, the

TABLE 1
CLINICAL DATA

Patient	*Age*	*Sex*	*Duration of symptoms*	*Cyanosis*	*Clubbing*	*Polycythemia*	*Congestive heart failure*	*Disability*	*Remarks*
				Primary diagnosis: Kyphoscoliosis					
T.C.	41	F	35 years	++	++	+	+++	++++	
N.V.	34	F	32 years	0	0	0	0	+	
				Primary diagnosis: Bronchiectasis					
H.H.	37	M	14 years	0	+	0	0	+	Moderate collaterals at operation.
M.S.	47	F	9 years	++++	+++	0	++++	++++	
G.C.	33	M	20 years	0	++	0	0	+	Extensive collaterals at operation.
E.V.	41	M	12 years	++++	+++	0	+	+++	
A.V.	30	M	23 years	0	0	0	0	++	
				Primary diagnosis: Asthma and emphysema					
P.M.	53	M	5 years	+	+	+	0	++	
A.W.	44	M	5 years	0	0	0	0	++	
				Primary diagnosis: Pulmonary tuberculosis					
F.B.	48	M	5 years	0	0	0	0	++	Post thoracoplasty; no collaterals seen in resected specimen.

absolute shunt, a_0, bore little relationship to the arterial saturation, whereas the algebraic sum of the right-to-left and left-to-right shunts of mixed venous blood, a, was somewhat better related to the saturation which decreased as a increased. Since the difference between a and a_0 is a function of the size of β, it would seem that the collateral flow serves in this way to minimize the effect of the poorly aerated lung on the arterial oxygen saturation.

In seven of the ten cases the presence of collateral flow could be determined by the present method (Table 3). Three of the ten cases came to surgery. In two of these the appearance of the resected lung specimen

after operation supported the estimation of collateral flow. In the third case coming to surgery no collateral channels were described in the pathological report. (No injection studies were done in this case.) This was in accord with the physiological studies in which no collateral circulation could be demonstrated.

The development of collateral flow has been ascribed to a high pressure gradient between the systemic and pulmonary arteries.[9] Such a condition is found in the tetralogy of Fallot in which extensive collateral vessels have been reported,[4] and in experimental pulmonary artery ligation in dogs.[8, 12] In the present cases, however, no such increased pressure gradient existed, and it is probable that chronic inflammation played an important rôle in the process. The finding of collateral circulation in cases of pulmonary arteriovenous fistulas without inflammatory changes has suggested that anoxemia itself may contribute to this process.[10] In the pulmonary cases reported here, anoxemia probably did not stimulate the development of the abnormal channels because there was not a significant correlation between the collateral flow volumes and the arterial oxygen saturations. However, as will be pointed out later, the highest arterial oxygen saturations occurred in the patients with the highest collateral flow volumes.

Although there seems to be no doubt that extensive precapillary anastomoses develop between the systemic arterial vessels and the pulmonary artery in chronic lung disease, the significance of these structures to the individual is equivocal. Similar collateral vessels have been described in cases of congenital heart disease of the cyanotic type.[4] In these congenital heart cases, the arterial unsaturation is chiefly due to admixture of venous blood with fully saturated pulmonary vein blood in the left heart chambers. The resulting unsaturated arterial blood is capable of further saturation with oxygen if it is recirculated through the pulmonary capillaries, since there is usually no increased "L" factor.[5] Therefore the collateral flow is of benefit to the individual by producing an increased volume of fully aerated blood.

In contrast to this, in the patients with chronic pulmonary disease the arterial unsaturation is the result of intrapulmonary factors. As it seems likely that the collateral circulation is prevalent (to the extent of a_0) in the diseased parts of the lung where further aeration is impossible, the recirculation of arterial blood to the lung through these collateral channels is a wasteful method of oxygen transport, and the burden of such a volume of ineffectual collateral circulation is borne by the left side of the heart which may, as a consequence of an increased work load, eventually fail.

Excluding the three patients in whom no collateral flow was found, the remaining seven may be divided into two groups; the first group of four patients whose resting arterial oxygen saturation fell below 85 per cent had consistently lower collateral flow values than the second group of three

TABLE 2
OBSERVED PHYSIOLOGICAL DATA

	Breathing room air							*Pressure, mm. Hg*	
Patient	*Arterial O_2 content vol. %*	*Dissolved O_2 vol. %*	*O_2 capacity vol. %*	*Saturation %*	*Venous O_2 content vol. %*	*Arterial O_2 content after breathing 100% O_2, vol. %*	*Oxygen consumed cc./min.*	*Right ventricle*	*Pul. Artery*
T.C.	16.4	0.14	20.1	80.9	11.3	20.2	210		17 mp.†
N.V.	17.7	0.23	18.7	93.4	12.7	19.7	149	9 mp	
H.H.	18.6	0.23	19.5	94.2	14.2	*20.6	227		24/7
M.S.	16.1	0.14	20.0	79.8	9.9	20.4	192	35 mp	
G.C.	15.6	0.18	17.6	87.7	12.4	18.3	219		32/19
E.V.	15.8	0.14	19.1	82.0	10.2	19.5	217	14/0	
A.V.	18.0	0.20	19.6	90.8	12.5	20.1	620		27/16
P.M.	17.7	0.13	22.3	78.7	12.6	22.5	271		47/25
A.W.	18.1	0.21	19.5	91.7	12.2	20.8	337		37/23
F.B.	16.3	0.19	18.0	89.4	10.9	19.1	346	24/10	

* The arterial O_2 capacity rose to 20.3 vol. % while breathing oxygen in the case of this patient.
† mp. = mean pressure.

whose resting arterial blood oxygen saturations were found to be above 85 per cent. This relationship between arterial oxygen saturation and collateral flow is a corollary of the difference between a and a_0 in their relation to the arterial oxygen saturation and may be a measure of the benefit the patient enjoys from the anastomoses between systemic and pulmonary arteries.

On postmortem examination, definite left ventricular hypertrophy has often been found in cases otherwise classified as having *cor pulmonale*.[11, 20, 21] This finding has been regarded with interest since the usual causes of high pulmonary artery pressure, in the absence of septal defects or mitral

TABLE 3
DERIVED PHYSIOLOGICAL DATA

Patient	*Decimal fraction* × *100*			SF	EPF	$\beta \times$ SF	*Range* β SF
	a	a_0	L	*L./min.*	*L./min.*	*L./min.*	*L./min.*
T.C.	12	15	15	4.1	3.62	1.06	0.600–1.700
N.V.	0	0	7	2.98	2.98	0	.....
H.H.	10	13.7	2	5.15	4.65	1.70	1.060–1.830
M.S.	8	8.8	17	3.1	2.87	0.56	0.272–0.975
G.C.	3	8.6	11	6.85	6.65	4.97	3.550–5.670
E.V.	7	9.7	15	3.9	3.61	1.11	.....
A.V.	4	8.35	7	11.3	10.9	6.75	.....
P.M.	11	13.6	18	5.3	4.75	1.67	.....
A.W.	0	0	10	5.7	....	0	.....
F.B.	0	0	11	6.4	....	0	.....

Symbols are defined in the text.

stenosis, have been thought to protect the left ventricle from strain. However, the effect of collateral circulation on the work load of the left ventricle has been referred to previously in regard to congenital heart disease,[22] and bronchiectasis.[19] In all probability the great burden imposed on the left ventricle by a large collateral circulation through the lung is an important factor in the paradoxical observation of left ventricular hypertrophy which has been reported in cases of *cor pulmonale* and cardio-pulmonary disease.

Summary

1. Physiological studies of the pulmonary circulation in ten cases of chronic pulmonary disease were presented.

2. In seven of the ten cases mixed venous blood was being shunted through non-aerated pulmonary capillaries.

3. In each of these seven cases collateral channels between the bronchial and pulmonary arteries were demonstrated.

4. These collateral channels were seen partially to overcome the anoxemic effect of venous blood being shunted through poorly aerated lung tissue into the pulmonary veins.

5. The volume of collateral flow required to affect favorably the arterial blood oxygen saturation was found to be excessively large.

6. This large volume of collateral flow, the burden of which is borne by the left ventricle of the heart, was suggested as a cause of the left ventricular hypertrophy often observed in cases of *cor pulmonale.*

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MOTOR NEURON DEVELOPMENT IN THE SPINAL CORD FOLLOWING REMOVAL OF THE ALAR PLATE AND SPINAL GANGLIA*

N. KARLE MOTTET

The number of motor neurons ultimately present in the anterior horn of the spinal cord of chick embryos is regulated by the volume of the tissue innervated. That this relationship between the number of motor neurons and the peripheral field is a causal one has been repeatedly demonstrated.[3, 6, 11] A reduction of the volume of periphery (i.e. by removal of wing or leg buds) results in a proportionally smaller number of motor neurons in the associated segments of the spinal cord. Furthermore, that intracentral factors (ascending and descending fibers) do not affect the number of motor neurons developed has been demonstrated by Danchaikoff and Agassiz[5] who transplanted segments of the medullary tube and adjacent tissue to the allantoic membrane and observed relatively normal spinal cord development. Employing a variety of techniques, Hoadley,[8] Bueker,[4] Levi-Montalcini,[9] and Hamburger[7] have confirmed this observation.

To define more precisely the nature of the relationship of the volume of the peripheral field to the development of motor neurons, Barron studied the histogenesis of the spinal cord of the sheep[1] and chick[2, 3] under normal and experimental conditions. Neuroblasts in the motor area were observed to become bipolar at the time that their axons reached the un-innervated periphery, and at the time that these neuroblasts became bipolar, those indifferent cells in close association with the dendrites of the bipolar cells began to develop axons (became unipolar). This led Barron to the following hypothesis: "That the peripheral field—the somatopleur—regulates the number of unipolar neuroblasts which continue their differentiation through the bipolar stage and the development of dendrites; that the dendrites in turn exercise an inductive influence on the associated indifferent cells which determines their course of differentiation into motor neuroblasts" (pages 93, 94).

More recently, additional evidence in support of this hypothesis was presented by Mottet,[10] who considered various mechanisms by which the periphery might regulate the ultimate number of motor neurons (i, the rate or duration of the production of the anlage—indifferent cells; ii, the differentiation of these cells; iii, the maintenance of neurons after differ-

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entiation). The results of his experiments were in full accord with the thesis that the periphery regulates the number of motor neurons by its induction of indifferent cells to differentiate into neurons. Thus a reduction in the volume of periphery results in a reduction in the number of indifferent cells which are induced to differentiate into neurons. However, whether or not the ingrowth of axons from the sensory areas to the motor area affects the number of motor neurons differentiated remains to be tested. The observations by Barron[8] and Mottet[10] that unipolar neuroblasts develop adjacent to the bipolar ones in the motor horn supports the view already presented—that the periphery exerts its inductive influence via the motor axons and cell bodies to surrounding indifferent cells. Concomitant with this, however, is the growth of sensory axons through the motor area; thus the possibility that the effect of changes in the volume of the periphery on the number of motor neurons is mediated via the sensory areas (spinal ganglion neurons, intramedullary neurons) has not been obviated.

To resolve this problem an experiment has been designed in which the sensory areas were removed in the lumbosacral region of the right half of the spinal cord before the neurons had differentiated. If the inductive influence of the periphery is mediated via the sensory areas, one would not expect to see motor neurons in the anterior horn; however, if it is by way of the motor axons, a normal motor horn would develop.

Materials and methods

LIST OF MATERIALS

Age of embryo	*Number of embryos*	*Motor neuron counts* *Number of embryos*
8	3	1
9	6	3
10	6	1
11	3	1
12	1	0
13	1	0

Twenty chick embryos from which the alar plate and spinal ganglia were removed from the right lumbosacral region of the spinal cord were prepared. Operations were performed after the embryos were incubated 60-70 hours. For a complete description of the operative technique the reader is referred to a previous report.[10] The alar plate and spinal ganglia were removed by making an incision in the midline through the dorsal commissure. The tension within the tissues is such that the dorsal portion of the cord then falls laterally, thus producing an experimental rachischisis. (If no further modification of the area ensues, the rachischisis will remain through the embryonic development and will appear in the adult.) The right alar plate and spinal ganglia of the lumbosacral region were then excised using a fine glass needle. It was necessary to avoid dissection lateral to the spinal cord because hemorrhage from the closely associated intersegmental arteries was almost invariably fatal. If, following the operation, a Speemann pipette is used to apply suction, the incision can be closed

and will heal (Fig. 2). However, some additional injury to the tissue is thereby incurred, so in most of the embryos the incision was left open (Fig. 1).

Embryos were killed at daily intervals from eight through thirteen days' incubation and fixed in Bouin's fluid. Serial sections were cut at 10 μ, stained with hematoxylin-erythrosin, and studied with the aid of a camera lucida. Motor neuron counts were made by drawing the nuclei, then totaling them after the drawings for an embryo were complete. Counts were made on every fifteenth section in six embryos and the results were in complete agreement. Because the operation did not affect the development of the contralateral side of the spinal cord, it (the left side) served as controls.

Observations

Specimen D 19 is a ten-day embryo from which seven consecutive right spinal ganglia were removed from the lumbosacral region along with the associated alar plate. The observations made on this embryo are representative of the entire series, therefore will be described in detail.

An alar plate, basal plate, and spinal ganglia were present on the control (left) side in the region of the operation, but only a basal plate was present on the affected side. Because of the operation the dorsal portion of the cord lay almost lateral to the ventral horn (Fig. 1). The epidermis was continuous with the surface of the spinal cord and, more medially, continuous with the ependyma. Some small round cells had accumulated near the exposed surface of the cord. The cartilage of the vertebra extend laterally, ending below the epithelium with a layer of mesenchyme interposed. Also there was an increased amount of mesenchyme separating the spinal cord from the vertebral cartilages.

On the control side a dense collection of neurons—the posterior sensory horn—occurred near the entrance of the sensory root fibers into the cord. The cells were sufficiently numerous to form a protuberance on the surface. The spinal ganglia had also developed normally. On the affected side the alar plate and spinal ganglia were absent, thus no sensory neurons were visible. Internuncial fibers extending toward the commissure, interspersed with neuroblasts, were present on the control side, whereas on the right side few neuroblasts and no internuncial fibers were seen.

In gross outline the motor horns appeared equal in size and shape bilaterally, as were the motor roots. When traced peripheralward the motor fibers, taking comparable courses bilaterally, were seen to extend into the muscles. Also the grey and white rami communicantes extended to and from the para-vertebral ganglia in a normal manner.

Motor neurons were counted in every fifteenth section by means of a camera lucida. A total of 397 motor cells was present on the affected side, whereas 407 were counted on the control side (a difference of 2½%).

In addition to the 20 embryos listed above, a seven-day embryo, 43 D, had the right half of the lumbosacral spinal cord removed and the incision closed. The ventral portion of the ependymal layer was apparently injured during the operation, thus the layer was discontinuous near the ventral

FIG. 1. Cross section through the spinal cord of embryo D 19 (x55). The side of alar plate and spinal ganglia removal is on the reader's left. Note the sensory and motor nerve roots, the sensory and motor horn neurons on the right (the embryo's left), and an equally large motor horn in the absence of sensory areas on the left (the embryo's right).

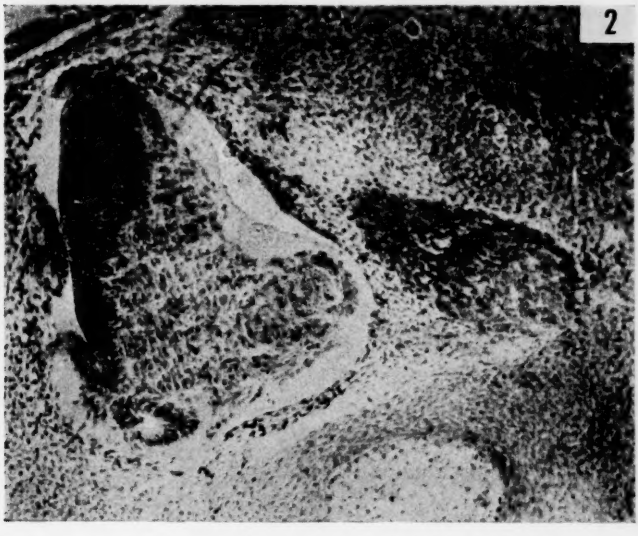

Fig. 2. Cross section through spinal cord of embryo 43 D (x165). The incision has healed after removal of right half of spinal cord. Note the innervation of the right periphery from the left half of spinal cord through the defect in the ependyma. More dorsally a space exists between the ependyma and mesenchyme.

commissure. In this region a few nerve fibers were observed to extend from the left anterior horn to innervate the right peripheral field (Fig. 2). These fibers were present only in the area where the ependyma does not form a continuous layer. Furthermore, the existence of a space between the mesenchyme on the right and the ependyma on the left suggested the possibility that some spinal fluid was present.

Discussion

The observations presented above, that removal of the sensory areas—the spinal ganglia and sensory horn—does not affect the motor neuron count, demonstrate that the inductive influence of the periphery on the differentiation of motor neurons is not mediated via the sensory areas. The number, size, and spatial relationships of the motor neurons are independent of the presence of the sensory areas.

This interpretation is warranted unless it be assumed that the presence of either intracentral or sensory fibers in addition to the periphery is necessary for the induction of indifferent cells to differentiate into motor neurons. Experimental removal of both intracentral and sensory areas in the same embryo has not been performed. To assume that either one is a necessary factor for the induction would be to state that the sum effect of both is equal to the effect of either factor. There is no evidence in support of this view. The oft-repeated experiments testing the intracentral factors and the volume of periphery on the number of motor neurons ultimately present in the spinal cord have been discussed in detail in a previous report,[10] and further description here is not necessary.

Summary and conclusions

The alar plate and spinal ganglia of the right lumbosacral region of 20 three-day chick embryos were removed. At daily intervals from eight through thirteen days' incubation, embryos were killed and fixed in Bouin's fluid. Serial sections were cut at 10μ and stained with hematoxylin-erythrosin. Removal of the sensory areas had no effect on the number of motor neurons in the anterior horn and there was no visible modification of their size, form, or spatial position. Also, the autonomic ganglia and fiber tracts developed normally. From this it was concluded that the induction by the peripheral field of indifferent cells to differentiate into motor neurons is not mediated via the sensory areas.

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AN UNUSUAL BRACHIAL PLEXUS FORMATION*

ALLEN CHETRICK AND L. R. M. DEL GUERCIO

The standard anatomy textbooks of today agree that the brachial plexus in man is derived from the anterior rami of the fifth, sixth, seventh, and eighth cervical and first thoracic nerves (Fig. 1). The anterior rami of the fifth and sixth cervical nerves are described as uniting at the outer border of the scalenus medius to form the cephalic trunk, the anterior rami of the eighth cervical and first thoracic nerves unite between the anterior and middle scaleni muscles to form the caudal trunk, while the anterior ramus of the seventh cervical alone represents the intermediate trunk. Each of the three trunks thus formed splits behind the clavicle into an anterior and a posterior division. The posterior divisions of all three trunks join to form the posterior cord and terminate in axillary and radial nerve branches. The anterior divisions of the cephalic and middle trunks join to form an outer cord, lateral with respect to the second part of the axillary artery and terminating in a musculo-cutaneous branch and the lateral root of the median nerve. The anterior division of the caudal trunk, however, remains single as the inner or medial cord, medial to the second part of the axillary artery. This cord ends by dividing into the ulnar nerve and the medial root of the median nerve.

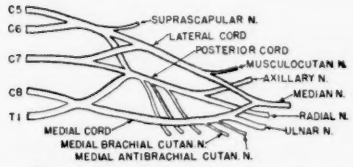

FIG. 1. Normal brachial plexus formation.

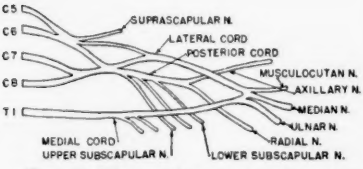

FIG. 2. First unilateral brachial plexus anomaly.

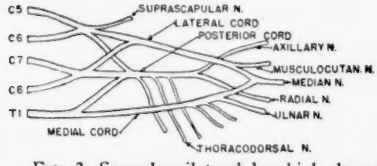

FIG. 3. Second unilateral brachial plexus anomaly.

Before this normal arrangement was statistically established, chiefly through the efforts of Herringham,[5] Lucas,[8] Walsh,[10] Harris,[3] and Kerr,[7] the form of the brachial plexus was one of the subjects in gross anatomy over which anatomists differed a great deal.

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Gray's Anatomy edited by Holmes in 1870 stated that "The fifth and sixth nerves unite near their exit from the spine into a common trunk. The seventh runs alone for some distance then joins the trunk near the outer border of the middle scalenus: and the three nerves thus form one large single cord. The eighth cervical and first dorsal nerves unite behind the anterior scalenus into a common trunk. Thus two large trunks are formed."[8]

Heath's *Anatomy* claimed: "The seventh nerve very frequently bifurcates, one branch going to the upper and one to the lower cord, being joined by branches from the other trunks."[4]

In the *Vade mecum* Wilson and Buchanan described the fifth and sixth nerves uniting to form one trunk, the last cervical and first dorsal nerve uniting to form another trunk, while the seventh, after running alone for some distance, divided into two branches which join the other cords. "At the point of junction the plexus consists of two cords, from which a third is given off and these become placed, one to the outer side of the brachial artery, one behind, and one to the inner side."[11]

J. F. Walsh in 1877 proposed that much of the variability in the plexus formation did not really exist, but was artificially produced.[10] He claimed that variations in which only two cords were described were manufactured by leaving pieces of fascia adherent between two of the three cords normally present. Conversely, variations in which the plexus was described as a mere interlacement of fibers with no definite plan were regarded by Walsh as instances where the dissection forcibly split up the nerves.

In any event, even the earlier anatomists apparently agreed that the fifth and sixth cervicals unite near their exit from the intervertebral foramina, likewise the eighth and first thoracic nerves, but the seventh runs singly for the greatest distance before dividing or uniting to the cephalic or caudal trunk.

Material and observations

Dissections of 30 cadavers and 60 brachial plexuses performed recently by medical students under the close supervision of instructors disclosed two unusual variations, apparently not recorded in the literature, in two cadavers, unilateral in each case yet slightly different.

The *first* anomalous brachial plexus arrangement consisted of a normal cephalic trunk, formed by the union of the anterior rami of the fifth and sixth cervical nerves; the middle trunk, however, was formed by the union of the anterior rami of the seventh and eighth cervical nerves under the anterior scalenus muscle—instead of the seventh cervical alone; also, the caudal trunk consisted of the first thoracic nerve alone instead of the normal union of the eighth cervical with it (Figs. 2 and 4). The first thoracic nerve or caudal trunk did not divide into anterior and posterior divisions, gave no contribution to the posterior, but formed the medial cord until it

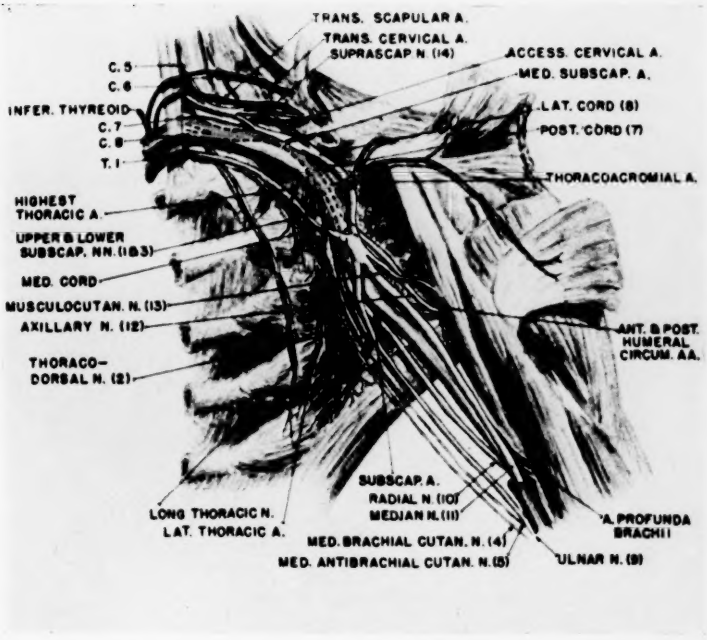

FIG. 4. Left (or *first*) unilateral brachial plexus anomaly.

branched into the ulnar nerve and the medial root of the median nerve. The intermediate trunk, with its uncommon union of the seventh and eighth nerves, divided into anterior and posterior branches. The posterior division joined the posterior division of the cephalic trunk to form the lateral cord which gave the usual branches and terminations, but which had an uncommon contribution from the eighth cervical nerve.

The *second* anomalous brachial plexus was similar* except for the contribution which the first thoracic nerve gave to the posterior cord (Fig. 3). The cephalic trunk was normal; the middle trunk was abnormal, for it consisted of the union of the seventh and eighth cervical nerves; and the caudal trunk was abnormal, since it comprised only the first thoracic nerve. The difference was that the posterior cord in Case 2 had contributions from all three trunks, for the first thoracic nerve, representing the caudal trunk, divided into anterior and posterior divisions and sent its posterior division to the posterior cord.

Discussion

Clinically, injury of the trunks of the brachial plexus would be of particular interest in either of these anomalous patterns. The so-called Duchenne-Erb paralysis, which affects muscles innervated by the fifth and sixth cervical nerves, would not be different from that in the normal plexus formation. In the middle type, however, with interruption of the middle trunk, one would expect to find more extensive involvement than normally occurring, since the eighth cervical nerve would also be included. Therefore, there would be not only more pronounced weakening of extensors of the arm and forearm and loss of cutaneous sensation in areas innervated by the seventh cervical nerve, but also (assuming that the eighth cervical nerve still supplied the muscles that it normally would, which was apparently the case) there would be a weakening of flexors of the fingers and loss of cutaneous sensation in those areas innervated by the eighth cervical nerve.

On the other hand, interruption of the lower trunk, Klumpe palsy, would be less extensive with the anomalous arrangements. In the normal plexus, muscle action supplied by the eighth cervical and the first thoracic nerves would be impaired. This presents a picture similar to combined ulnar and median palsy with defects chiefly in finger flexion and adduction and general wrist movements. Without eighth cervical nerve involvement, however, and since the eighth cervical nerve in the anomalous patterns was seen to contribute to the median and ulnar nerve formations, the disabilities of lower trunk injury would be less severe.

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* This anomaly was found on the right side, but it has been drawn as a left plexus in Figure 3 to facilitate comparison with Figures 1 and 2.

Summary

Two anomalous brachial plexuses were observed in the dissection of 30 cadavers. A variation from the normal seen in both was the formation of the medial cord by the first thoracic nerve alone.

In one instance the first thoracic nerve failed to contribute to the posterior cord. There is no previous recording of this anomaly.

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CHORIONIC GONADOTROPHIN, ACTH, AND THE ADRENAL-HYALURONIDASE RELATIONSHIP*

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It has been shown that either adrenocorticotrophic hormone (ACTH) or the 11-oxygenated adrenal cortical steroids inhibit the hyaluronidase-enhanced spreading phenomenon in the rabbit and in the mouse.[7, 8, 9]

In view of the favorable influence of pregnancy on certain of the collagen diseases, it was of interest to determine whether chorionic gonadotrophin had any influence on this phenomenon. This was found to be the case, but in the course of these investigations it became apparent that the inhibition produced by this agent was probably due, not to the gonad-stimulating principle, but to a contaminant which exhibited many of the properties previously associated with ACTH from pituitary tissue.

While this work was in progress, Jailer and Knowlton[4] published a paper in which the presence of ACTH-like activity in extracts of placenta was indicated. The present study is to be regarded as of a preliminary nature since many points concerning the nature of the adrenal cortical stimulating principle remain to be clarified.

Materials and methods

Detailed methods and precautions as to procedures have been described in earlier publications.[6, 7] Male and female mice of the inbred CBA strain, 10 to 12 weeks of age and weighing an average of 25 to 30 gm. were used in these investigations.

The Wyeth standardized hyaluronidase preparations used in these studies were designated W-108-A and W-160-A; the latter enzyme preparation was approximately three times as potent as the former.

The ACTH preparations were from the Armour laboratories. (Earlier experiments, using Lot H-7911 in a variety of experimental conditions, gave anomolous results, presumably due to the high oxytocin and pressor activity present.) The preparation used in most of these studies was Lot H-8412 and contained 0.2 unit oxytocin and 0.14 unit pressor substance. The contents of one vial were noted to be equivalent to 35 mg. of Armour Standard LA-1-A.

The chorionic gonadotrophin (CG) and the heat-inactivated chorionic gonadotrophin (HICG) preparations from human pregnancy urine were supplied by Dr. R. H. Barnes and Dr. R. G. Westfall of Sharp & Dohme, Inc. They were prepared by the method of Gurin[5] and assayed in the 18-day albino rat, the criterion of response being the results of vaginal smears at 100 hours following subcutaneous injection. Preparations of varying gonadotrophic potency were tested: (1) = 400 U/mg.; (2) = 785 U/mg.; (3) = 330 U/mg. The HICG was prepared by heating a water solution of CG for 15 min. at 100° C. in a boiling water bath, followed by cooling and lyophilization. It is

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noteworthy that such a procedure does not inactivate ACTH preparations.* However, ACTH activity can be lost in such a procedure if the degree of acidity is not carefully controlled.

The CG and HICG preparations were dissolved in sterile physiological saline just prior to experimental use. Where administered in large doses, the question of the toxicity of certain other CG preparations tested is of importance. It is quite possible that lack of precautions exercised initially in the collection of urine and in the isolation of the gonadotrophins might result in bacterial contamination; consequently, pyrogenic effects might be noted with their use. This was not the case in the samples tested and here, in part, reported. Although in adrenalectomized mice tested six days postoperatively, dosages of ACTH, CG, and HICG in excess of 3.5 mg. proved to be toxic, this was found not to be true in the normal animal. Dosages ranged from 0.5 to 5 mg. in the various investigations and are cited in detail in the individual experiments. Control groups were injected with equal amounts of saline.

Bilaterally adrenalectomized animals were tested one and six days postoperatively. They were not maintained on saline.

Groups of castrated animals of both sexes were used four and 10 days postoperatively. It is interesting that in these experiments the males and females exhibited a slight sex difference in hyaluronidase-enhanced spreading areas, and that castration alone appeared to inhibit to a slight degree this spreading.

The hypophysectomized mice† were 12 weeks of age, of both sexes, and weighed 25 to 30 grams. They were maintained on 5 per cent glucose and were tested forty-eight hours postoperatively. Autopsies on all animals reported here showed the completeness of hypophysectomy. Only a slight degree of adrenal atrophy occurred in the period prior to the experiment while the weight loss was between 1 and 2 grams.

The previous practice of carrying out comparable bilateral intradermal injections of saline and India ink and of hyaluronidase and India ink has been employed in most of the studies presented here. However, in the assay procedure, since the major effect of the inhibition has been shown to be on the exogenously added hyaluronidase, the tables have been set up uniformly to give information on the hyaluronidase-spreading areas.

The rats employed in the assay for ACTH activity were males of the Sprague-Dawley strain. Hypophysectomized animals‡ weighing between 180 and 200 grams were used on the third postoperative day. Under hexobarbital or light sodium pentobarbital anesthesia one adrenal gland was removed before and the other one hour after 0.2 ml. of the test material was introduced into the inferior vena cava. Tail blood samples were obtained at the same time intervals. Eosinophile counts and ascorbic acid were determined as described in an earlier paper.[1] Intraperitoneal injections were made on unanesthetized animals.

Experimental

The systemic administration of ACTH, CG, or HICG in dosages ranging between 1 and 5 mg. produced a marked inhibition of the dermal spreading of India ink with hyaluronidase in normal animals. Results of some representative experiments are show in Table 1. The similarity of action between

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* Li, C. H. and Evans, H. M.: *Vitamins and hormones. Advances in research and applications.* New York, Academic Press, Inc., 1947. Vol. V, p. 220.

† We are indebted to Dr. W. U. Gardner and to Dr. J. H. Trentin, Department of Anatomy, Yale University School of Medicine, for the preparation of most of these animals.

‡ Obtained from Hormone Assay Laboratory, Chicago, Illinois.

ACTH and CG, in terms of percentage inhibition, is clearly seen, and while the known impurity of both CG and ACTH preparations makes it impossible to quantitate dosages, the effects noted are qualitatively identical. The inhibition of spreading appeared to be maximal in the dosage ranges used, and it is of interest that preparations of known varied gonadotrophic potency produced this similar and constant degree of inhibition of spreading. Further, that while extremely large doses were purposely tested, no symptoms of toxicity resulted in the normal mice. Control studies using serum albumin in similar dosages were without inhibitory effect.

TABLE 1

COMPARISON OF EFFECTS OF ACTH, CHORIONIC GONADOTROPHIN, AND HEAT-INACTIVATED CHORIONIC GONADOTROPHIN IN MICE

Substance injected	*Dose* mg.*	*Route of administration*	*Time† interval hours*	*Area of spreading with hyaluronidase and India ink*		
				Control (saline) sq. mm.	*Experimental (test substance) sq. mm.*	*% Change from control*
			Normal mice			
ACTH	1.0	i.p.	3	418 (12)	265 (12)	—37
ACTH	3.5	i.p.	3	418 (12)	205 (12)	—51
CG (1)	1.0	i.p.	3	400 (12)	247 (12)	—38
HICG (1)	1.0	i.p.	3	368 (12)	190 (12)	—48
CG (2)	2.0	i.p.	3	390 (15)	211 (12)	—46
CG (3)	2.0	i.p.	3	390 (15)	185 (12)	—53
CG (1)	3.5	i.m.	3	400 (12)	208 (12)	—48
CG (1)	5.0	i.m.	3	354 (12)	207 (12)	—41
HICG (1)	5.0		3	354 (12)	197 (12)	—44
			Adrenalectomized mice			
ACTH	1.0	i.p.	3	645 (12)	638 (12)	0
ACTH	3.5	i.p.	3	645 (12)	694 (12)	+7
CG (1)	3.5	i.p.	3	687 (12)	672 (12)	—2

CG (1) = 400 u/mg.
CG (2) = 785 u/mg.
CG (3) = 330 u/mg.
* Diluent equals 0.5 cc. physiological saline.
† Interval between injection of steroids and hyaluronidase.

The fact that HICG gave entirely similar results in terms of degree of inhibition produced was of importance in that the procedure of heat-inactivation destroyed essentially all gonadotrophic activity, but it has been noted that under similar conditions ACTH activity is not destroyed.*

* Li, C. H. and Evans, H. M.: *Op. cit.*, vol. V, p. 220.

Numerous experiments showed that CG and HICG were completely without effect in the adrenalectomized animal. Their inhibitory action on hyaluronidase appeared to be mediated through the adrenal,† as was found in similar experiments employing ACTH. One to 3 mg. doses of these three substances were well tolerated, but dosages of 5 mg. of HICG, CG, or ACTH proved fatal to adrenalectomized animals. The fact that certain other CG preparations proved toxic to the adrenalectomized animal in smaller dosages led to further investigations.

TABLE 2

INFLUENCE OF CHORIONIC GONADOTROPHIN AND HEAT-INACTIVATED CHORIONIC GONADOTROPHIN ON CASTRATED MICE AND NORMAL MICE

Substance injected	*Dose† mg.*	*Route of administration*	*Time interval hours*	*Area of spreading with hyaluronidase and India ink**		
				Control (saline) sq. mm.	*Experimental (test substance) sq. mm.*	*% Change from control*
Normal female mice						
CG (1)	3.5	i.p.	3	642 (12)	262 (12)	—59
Castrated female mice						
CG (1)	3.5	i.p.	3	502 (12)	210 (12)	—58
Normal male mice						
HICG (1)	3.5	i.p.	3	580 (12)	203 (12)	—65
Castrated male mice						
HICG (1)	3.5	i.p.	3	543 (12)	217 (12)	—60

* Enzyme preparation W-160-a. Potency approximately 3x that of W-108-A. Spreading time of 6 hours.
† Diluent 0.5 cc. saline.

Experiments in normal animals wherein hyaluronidase spreading was allowed to continue over time intervals of one to six hours showed that the inhibition appeared almost maximal at one hour, thus indicating a rapid and marked stimulation of the adrenal with subsequent release of C-11 adrenal steroids resulting in an almost total inhibition of enzyme activity.

The fact that ACTH, HICG, and CG preparations of different gonadotrophic potency produced similar inhibitory effects on hyaluronidase activity, and that none of these effects was seen in the adrenalectomized animal, showed similarity of action between CG, HICG, and ACTH.

† When administered locally at the intradermal site of injected hyaluronidase, these preparations showed no inhibitory effect in either normal or adrenalectomized mice. Similarly, in *in vitro* studies, CG, HICG, and ACTH did not inhibit hyaluronidase.

Earlier work has shown that the sex steroids, progesterone, pregnenolone, estradiol benzoate, and testosterone are without effect in the normal and adrenalectomized animal.[5,9] However, the possibility remained, as pointed out by Greep,[2] that CG may stimulate the secretion of substances other than estrogens and androgens. The role of the gonads in this relationship was then investigated.

In Table 2 are seen the results of representative experiments showing the very marked inhibitory effects produced by the administration of CG and HICG to groups of castrated animals of both sexes. Control groups of castrated animals were injected with physiological saline. In the results here summarized, a high-potency enzyme preparation and increased

TABLE 3

EFFECT OF CHORIONIC GONADOTROPHIN IN HYPOPHYSECTOMIZED MICE

				Area of spreading with hyaluronidase and India ink		
Substance injected	*Dose mg.*	*Route of administration*	*Time interval hours*	*Control (saline) sq. mm.*	*Experimental (test substance) sq. mm.*	*% Change from control*
CG (2) males	1	i.p.	3	414 (6)	215 (5)	—48
CG (2) males	3	i.p.	3	414 (6)	190 (5)	—54
CG (1) females	3	i.p.	3	538 (6)	232 (5)	—57
HICG (1) females	3	i.p.	3	538 (6)	135 (5)	—75

hyaluronidase-spreading time were employed to determine whether there would be equal effectiveness in inhibitory capacity with respect to enzyme potency. Results show that potency of enzyme or time interval of spreading was not a limiting factor in CG inhibitory capacity. The presence or absence of the gonads exerted no effect in this investigation; in fact, the absence of the gonads appeared slightly to inhibit hyaluronidase spreading in control castrated as compared with normal animals. It is interesting that the absolute areas of spreading and degree of percentage inhibition produced were nearly constant in animals receiving CG and HICG.

The obviously critical point in evaluating the ACTH-like activity of CG and HICG was the establishment of what effects would be shown by the administration of these preparations in the absence of the pituitary. The results of experiments with 48-hour hypophysectomized mice are given in Table 3. It is clearly seen that CG and HICG inhibit the hyaluronidase-enhanced spreading phenomenon even in the absence of the pituitary.

It appears from the data presented in the foregoing tables that the area of spread resulting from the inhibition of hyaluronidase by ACTH, CG, or HICG is relatively constant. Earlier investigations[8] and, more recently,

studies with the pure steroids F, E, and A, employing *in vitro* as well as *in vivo* methods yielded a similar degree of constancy.[8] It has been concluded that the area of spreading under these conditions is minimal and represents essentially complete inhibition of the enzyme hyaluronidase. The percentage inhibition presented in the tables is a relative figure and because of the method of calculation (in which there is always a certain degree of spreading when saline and India ink intradermal injections alone are employed) it is apparent that in these terms, 100 per cent inhibition would be impossible to obtain. However, on a theoretical basis it is likely that these substances have brought about approximately complete inhibition. This same marked inhibition of hyaluronidase has been evident in experiments with the pure steroid Compounds E and F. One may assume that the

TABLE 4

ASSAY OF ACTH ACTIVITY OF CHORIONIC GONADOTROPHIN IN THE HYPOPHYSECTOMIZED RAT

Rats	*Chorionic gonadotrophin* mg./100 g. body wt.*	*Adrenal % change in ascorbic acid* *mg./100 g.*	*total mg.*	*Blood % change in eosinophiles*
29 Hypox. 3 days	.....	—2 ± 1.4	+ 3 ± 1.7	....
8 Hypox. 3 days	2, intraven.	—7 ± 1.9	—13 ± 1.6	—74
2 Hypox. 4 days	1, intraper.	.....	.....	—45

* Preparation #1 (400 U/mg.).

C-11 oxygenated steroids that are released as a result of ACTH or CG administration are quantitatively similar in their effects to a dosage of Compounds E and F that have been found to bring about the same marked inhibition of the enzyme.

Additional tests of the ACTH activity of CG 1 were done in rats, where a 60 per cent reduction of the adrenal ascorbic acid and circulating eosinophiles was observed in normal and castrated male animals injected with 4 mg./100 g. of CG intraperitoneally. The action of CG in rats whose hypophyses had been removed provided a somewhat better measure of the adrenocorticotrophic action of this preparation; results of these experiments compared with a series of operated controls are recorded in Table 4. Hypophysectomized rats responded to intraperitoneal injections of CG, 1 mg./100 g., with marked eosinopenia in one hour. With intravenous administration the decrease in blood eosinophiles far exceeded the per cent fall in adrenal ascorbic acid. Since there is no evidence that eosinopenia can be produced in one hour in the hypophysectomized rat either by surgical trauma or agents other than ACTH,[8] the reduction in eosinophiles and the small but significant decline of the ascorbic acid in the adrenal,

effected by CG, support the conclusions obtained from the studies of its inhibition of the hyaluronidase action in the mouse.

Discussion

Several similarities in the biologic activity of ACTH and CG from human pregnancy urine have been shown. Furthermore, it has been demonstrated that CG exhibits ACTH-like effects that cannot be associated with its gonadotrophic activity.

The following evidence establishes this similarity in action: both CG and HICG cause a marked inhibition of hyaluronidase-enhanced spreading in normal and castrated animals in the presence of the adrenals but are without effect in the adrenalectomized animal. The possibility that this effect was due to non-specific toxicity or mediation through the gonads has been ruled out insofar as it was possible. This effect was qualitatively identical to that found with ACTH. In the hypophysectomized animals, both CG and HICG were found to exhibit the same marked degree of inhibition of hyaluronidase activity.

There are two possibilities as to the source of the ACTH-like activity found in these preparations from human pregnancy urine. The first is that the process of extraction includes such quantities of pituitary ACTH as may have been excreted in the urine; the second is that the placenta may normally form an ACTH-like agent, a portion of which is also excreted in the urine. These experiments provide no means of discriminating between the two possibilities; however, the recent report of Jailer and Knowlton[4] that ACTH-like activity can be demonstrated in placental tissue indicates that the urinary factor demonstrated by these experiments might have a similar origin.

These investigations suggest the possibility that ACTH or ACTH-like material of human origin may be obtained for clinical use, thus providing another source to supplement the limited quantities of this agent now available only from animal sources.

Summary

1. Chorionic gonadotrophins (CG) and heat-inactivated chorionic gonadotrophins (HICG) isolated from human pregnancy urine caused a marked inhibition of hyaluronidase-enhanced spreading in normal and castrated mice in the presence of the adrenals and were without effect in the adrenalectomized animal. Furthermore, this effect was qualitatively similar to that found with ACTH.

2. The possibility that this inhibitory effect on hyaluronidase-spreading was due to non-specific toxicity, or mediation through the gonads, was ruled out insofar as possible.

3. In hypophysectomized mice, both CG and HICG were found to

exhibit the same marked degree of inhibition in hyaluronidase-enhanced spreading.

4. The presence of ACTH activity in the CG preparation was indicated by the eosinopenia and reduction of adrenal ascorbic acid observed in the hypophysectomized rat.

5. Evidence is presented showing similarity of action of ACTH, CG, and HICG with respect to the specific biological effects investigated.

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THE ACTION OF PENICILLIN ON THE GROWTH RESPONSE OF A GRAM-NEGATIVE BACILLUS TO AMINO ACIDS AND PEPTIDES*

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Since peptides are structural intermediates between amino acids and proteins, they have also been assumed to be metabolic intermediates in the biosynthesis of proteins from amino acids. Experimental evidence for this view is meager, and, for this reason, it appeared desirable to conduct systematic studies of the metabolism of peptides in microorganisms. In particular, an effort has been made to find organisms whose protein synthesis, as measured by the extent of growth, is promoted more effectively by a given peptide than by the amino acids of which the peptide is composed. Such an organism was found as a contaminant in a solution of L-leucylglycine in 0.9 per cent sodium chloride. As reported previously,[10] this organism (strain SF) does not grow to an appreciable extent in the presence of an equimolar mixture of L-leucine + glycine, dissolved in saline. In a more complex basal medium, containing inorganic phosphate, growth occurs in the presence of a mixture of leucine + glycine; the lag period, however, is longer than that observed with an equivalent amount of leucylglycine.[11] It was of interest to find that, in the more complex basal medium, the growth of strain SF in the presence of the amino acid mixture was inhibited by penicillin at concentrations which did not exert an inhibitory effect in the presence of the peptide.[11]

Several investigators have examined the relationship of penicillin action to the amino acid metabolism of both Gram-positive and Gram-negative organisms. Shwartzman[9] has reported that the sensitivity of certain Gram-negative bacilli cultured in a salt-glucose medium is counteracted to a marked degree by dicarboxylic and basic amino acids and by cystine. Gale[3, 4, 5] has found that Gram-positive cocci grown in the presence of penicillin lose their ability to remove glutamic acid from the medium. This effect has been attributed to an interference with the mechanism by which certain amino acids are taken into the cell, and to the inhibition of the "formation of a substance whose synthesis is essential for the assimilation process to occur."[4] Hotchkiss[7] has reported that the antibiotic prevents the synthesis of protein by non-proliferating cells of *Staphylococcus aureus* and causes the production of extracellular material presumed to be a polypeptide. Badger and Krampitz[1] have reported that the addition of penicillin

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to resting cells of *S. aureus* in a medium containing C^{14}-glycine greatly reduces the incorporation of C^{14} into the cellular nucleic acids. The data reported previously[11] and the experiments presented in this communication demonstrate that penicillin alters the nitrogen metabolism of strain SF, and has a specific effect upon the assimilation of glycine by cultures in the lag phase of growth. The hypothesis has been offered[11] that glycine assimilation by strain SF involves the prior incorporation of the amino acid into a peptide, and that the bacteriostatic action of penicillin is due to an inhibition of peptide synthesis.

Strain SF is a motile, polar flagellated, and predominantly monotrichous, Gram-negative bacillus. The motility is best observed in cultures incubated at about 20°; cultures grown at 37° are practically non-motile. The organism liquefies gelatin, and turns litmus milk alkaline. It forms neither acid nor gas from glucose, lactose, or sucrose. The final pH of the media in the fermentation tests is 8 or higher. In a synthetic medium composed of inorganic salts, including phosphate and ammonium ions, the bacteria can utilize glucose, glycerol, and acetate, but not citrate or formate, as carbon sources. In the absence of phosphate, growth does not occur on glucose and ammonium ions. At the suggestion of Dr. Einar Leifson,* Loyola University School of Medicine, strain SF has been classified as a member of the genus *Pseudomonas*, rather than of the genus *Alcaligenes* as suggested earlier.[10]

Methods

The basal medium employed in these studies contained, per liter, 5 gm. NaCl, 0.2 gm. $MgSO_4 \cdot 7H_2O$, 2 gm. K_2HPO_4, 1 gm. KH_2PO_4, and 1 ml. of a solution of trace elements.[6] The pH of this medium is about 6.8. All tests were carried out in Evelyn colorimeter tubes containing the basal medium supplemented with the desired concentrations† of test compounds (final volume 10 ml.) and sterilized in the autoclave for 15 minutes at 15 lbs. pressure. When glucose, or other carbon sources were tested, the solution of the compound was autoclaved separately, and aliquots were added to the sterile medium. The inocula were prepared as aqueous suspensions of cells harvested from a 24-hour, peptone-yeast extract-agar slant. Approximately 10^8 cells, in 0.5 ml., were added to the medium in each colorimeter tube, except where indicated otherwise. After inoculation, the tubes were incubated, in a slanted position, on a shaker at 30°, and the extent of bacterial growth was measured turbidimetrically, at intervals of 6 to 12 hours, in an Evelyn colorimeter equipped with filter No. 540. It has been found that the optical density of such cultures is directly proportional to the number of viable cells and to the protein-nitrogen content of the cultures.

The response of strain SF to the various amino acids and peptides tested was found to vary with respect to the maximal extent of growth, to the duration of the lag period (the interval between the time of inoculation and the appearance of measurable turbidity—*ca.*, 4×10^8 cells per 10 ml.), and to the generation time (the time required

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† In what follows, the concentration of each test compound will be given in moles per liter of medium.

for the doubling of the optical density of a culture during logarithmic growth). In the sections to follow, the response to each test compound is expressed in terms of these three characteristics of the resulting growth curve. This method of presenting the data is illustrated in Table 1, which describes the growth response of strain SF to 0.005 M leucylglycine, leucine + glycine, leucine, or glycine.

In the experiments in which penicillin (penicillin G sodium salt—Pfizer) was used, it was made up in a filter-sterilized, aqueous solution and was added to the sterile media immediately prior to inoculation.

TABLE 1

A REPRESENTATIVE EXPERIMENT SHOWING THE GROWTH RESPONSE OF STRAIN SF

Test compound	*Lag period*	*Generation time*	*Final optical density**
	hours	*hours*	
L-Leucylglycine (0.005 M)	8	8	0.44
L-Leucine (0.005 M) + glycine (0.005 M)	14	8	0.46
L-Leucine (0.005 M)	20	20	0.43
Glycine (0.005 M)	30	8	0.071

* A tube containing sterile medium has an optical density of 0.

Results—Growth of strain SF in glucose-free media

Growth response to L-leucylglycine. The maximal growth attained by strain SF in the basal medium supplemented with leucylglycine (0.008 M or higher concentrations) corresponded to an optical density of about 0.60 (*ca.*, 2×10^{10} cells per 10 ml.). At concentrations below 0.008 M, the extent of growth was a function of the initial concentration of leucylglycine. The duration of the lag period and the generation time observed in the presence of leucylglycine (0.001-0.04 M) were the same at all concentrations. Although 5-100 Oxford units of penicillin per ml. had no effect on the growth response to leucylglycine, a slight prolongation of the lag period was observed with 500 Oxford units. In the latter case, however, some killing of the cells occurred during the lag period.[11]

Growth response to equimolar mixtures of L-leucine and glycine. In a medium containing an equimolar mixture of leucine (0.005 M) + glycine (0.005 M), the lag period was longer than that for leucylglycine (0.005 M); however, the lag period for the amino acid mixture was shorter than that for leucine (0.005 M) or for glycine (0.005 M) (Table 1). This relationship held true for all concentrations of the above compounds in the range of 0.001-0.01 M.

The lag periods in media containing equimolar mixtures of leucine + glycine, at concentrations of 0.001-0.008 M, were approximately the same,

but, above 0.008 M, the lag period was markedly greater, and, at 0.04 M, no growth was observed. The maximal optical density and, to some extent, the growth rate were increased as the concentration of the amino acids was raised from 0.001 M to 0.008 M. However, at the 0.008 M level, the growth curve had a definite diphasic character; the curve rose rapidly at first and, after a short period during which the slope was greatly reduced, a second rapid rise was seen. The slope of the second rise in the growth curve was less steep at a concentration of 0.01 M than at 0.008 M, and, at 0.02 M, there was no second rise. At 0.02 M, the final optical density of the culture was only about half that for the 0.005 M level.

When penicillin (5 Oxford units per ml.) was added to the medium containing the amino acid mixture at a concentration of 0.005 M, a marked prolongation of the lag period was noted.[11] At a lower amino acid concentration (0.001 M), penicillin exerted no measurable effect, but the inhibitory action of penicillin became more pronounced as the amino acid concentration was increased, reaching a maximum at the 0.005 M level. Despite its effect on the lag phase of growth, the antibiotic did not alter the generation time. Earlier studies[11] had shown that, at a level of 5 Oxford units per ml., penicillin had no appreciable bactericidal effect under these experimental conditions.

Growth response to L-*leucine.* In contrast to the relatively limited growth obtained with high concentrations of leucylglycine, the extent of growth on leucine increased as the initial concentration of the amino acid was raised from 0.001 M to 0.05 M. At the latter concentration, the optical density attained was about 1.10 (4×10^{10} cells per 10 ml.). However, in the concentration range 0.001-0.008 M, leucine produced growth equal in extent to that caused by equivalent concentrations of leucylglycine, or of a mixture of leucine + glycine.

It is of interest that the carbon and nitrogen of leucine (0.005 M) are utilized for growth to the same extent as is carbon and nitrogen supplied in the form of glucose (0.005 M) and ammonium chloride (0.005 M). Both the lag period and the generation time are much shorter in the glucose-ammonium chloride medium (11 and 4 hours respectively) than in the leucine medium.

The addition of as little as 0.001 M glycine to a medium containing leucine at any concentration tested shortened both the lag period and the generation time characteristic of growth in the presence of leucine alone. However, the presence of ammonium chloride (0.001-0.005 M) in a medium containing leucine did not alter the growth response to the amino acid. The stimulating effect of glycine was not due, therefore, solely to its function as a readily available source of nitrogen.

The effect of penicillin on the growth response of strain SF in media containing leucine alone was similar to that noted with leucylglycine; the antibiotic also had no effect on the growth in the glucose-ammonium

chloride medium. The inhibitory action of penicillin in a medium containing a mixture of leucine + glycine has been ascribed, therefore, to a specific effect upon the ability of the organism to assimilate free glycine.[11]

Growth response to glycine. In the presence of glycine as the sole source of carbon and nitrogen, the growth of strain SF was less than that noted with equivalent amounts of leucylglycine, leucine, or a mixture of leucine + glycine (Table 1). Even when the extent of growth produced by these nutrients was compared on the basis of the carbon supplied by each test compound, glycine was found to be utilized much less efficiently than were either leucylglycine or leucine. At concentration levels from 0.001 to 0.02 M, glycine permitted growth up to an optical density of 0.17. However, at 0.04 M glycine, no growth was observed, thus showing that, at high concentrations, this amino acid inhibits the growth of strain SF. In general, the duration of the lag period in the presence of glycine was increased as the initial concentration of the amino acid was raised. At a concentration of 0.005 M, the lag period was about 30 hours; at 0.02 M, it was 90-100 hours.

Five Oxford units of penicillin had no inhibitory effect in the presence of 0.001 M glycine, but, at glycine concentrations of 0.003 M or higher, penicillin inhibition was very marked. The most pronounced prolongation of the lag period by penicillin was observed with 0.005 M glycine. Concentrations of penicillin above 5 Oxford units per ml. caused relatively more inhibition, but also resulted in the killing of a large proportion of the bacteria. The bactericidal action of high concentrations of penicillin was always much greater in media containing glycine, or glycine supplemented with leucine, than in media containing leucylglycine or leucine.

Utilization of the nitrogen of glycine for protein synthesis. It appeared of interest to determine the relative extent to which the nitrogen atoms of leucine and of glycine were utilized for protein synthesis, either when supplied in the form of leucylglycine or in the form of a mixture of the two amino acids. For this purpose, use was made of N^{15} labeled glycine,[8] containing 32.366 atom per cent excess N^{15}, and of L-leucylglycine, containing 31.432 atom per cent excess N^{15} in the glycine-nitrogen. The isotopic peptide was synthesized in the manner described previously for the non-isotopic compound.[2] The isotopic leucylglycine and a mixture of leucine + isotopic glycine were dissolved in the basal medium, which was then dispensed, in 10 ml. volumes, into Evelyn colorimeter tubes. After the usual sterilization and inoculation procedures, the culture tubes were incubated on a shaker at 30°. In Experiment I, the cultures were allowed to grow to completion (optical density *ca.* 0.43-0.48); in Experiment II, culture tubes were removed from the incubator at intervals so the N^{15} content of the bacterial proteins could be measured at stages in the logarithmic phase of growth, *i.e.*, when the optical density was approximately 0.1 or 0.25.

As soon as the culture tubes were taken from the incubator, trichloroacetic acid was added to each tube to give a 6 per cent solution. In order to provide sufficient protein-nitrogen for the N^{15} analyses, the contents of several tubes were pooled and worked up together. After centrifugation, the trichloroacetic acid precipitate was resuspended in a 6 per cent trichloroacetic acid solution, left in the ice box overnight, and then was washed several times more with 6 per cent trichloroacetic acid. In Experiment I, in which two sets of tubes were used for each test medium, the precipitate from one set was washed 3 times, and that from the other set, 4 times. N^{15} analyses showed that 3 washings were sufficient to free the precipitated proteins from soluble compounds containing isotopic nitrogen. In Experiment II, therefore, the trichloroacetic acid precipitates were washed only 3 times. The washed proteins were at once suspended in approximately 50 per cent sulfuric acid, and the nitrogen content of the material was determined by the Kjeldahl method. At maximal growth, the bacterial protein-nitrogen amounted to about 17 per cent of the nitrogen supplied in the media in the form of leucylglycine or as the mixture of leucine + glycine.

The ammonia obtained in the Kjeldahl-nitrogen determination was analyzed for N^{15}. In Experiment I, the isotopic ammonia from the bacterial proteins was analyzed directly; in Experiment II, the ammonia was diluted with a known amount of non-isotopic ammonia prior to the N^{15} analysis. The results of these experiments are given in Table 2. The data in the last column of the table show that the cell proteins always contained more leucine-nitrogen than glycine-nitrogen, *i.e.*, less than 50 per cent of the protein-nitrogen was derived from the glycine in the medium. The isotope content of the protein from cells grown on the dipeptide and on the amino acid mixture did not change appreciably during the period of growth in these experiments. Thus, despite the fact that glycine stimulates the growth response of strain SF to leucine, the nitrogen of glycine was not used preferentially during the period up to the time of one-fourth maximal growth.

The data also suggest that more glycine-nitrogen was used for protein synthesis when the cells were supplied with free glycine than when they received leucylglycine. While the differences between the N^{15} utilization of leucylglycine and of glycine supplemented with leucine were not very great, the utilization of the nitrogen of free glycine was higher in every case. The difference was evident even when the cells from a medium containing isotopic leucylglycine, non-isotopic glycine, and leucine were compared with cells from a medium containing leucylglycine, isotopic glycine, and leucine.

An experiment with isotopic glycine and leucylglycine also was carried out in an attempt to determine the effect of penicillin on the incorporation, into the bacterial proteins, of glycine-nitrogen supplied either in the free

amino acid or in the peptide. This test was run as part of Experiment II, and was identical with those described above except for the presence of 5 Oxford units of penicillin per ml. of medium. The data obtained are given

TABLE 2

N^{15} EXPERIMENTS WITH STRAIN SF GROWN IN GLUCOSE-FREE MEDIA

Exp. no.	*Test compound**	*Optical density of culture*	N^{15} *Analysis*			*Protein-N derived from glycine-N of media†*
			Dilution of sample	N^{15} *in diluted sample*	N^{15} *in cell protein*	
				atom % excess	*atom % excess*	%
I	L-Leucylglycine*	0.43	0		11.655‡	37.1
I	(0.005 M)	0.46	0		11.525§	36.7
II		0.25	3.189	3.469	11.06	35.2
II		0.099	2.735	4.059	11.10	35.3
I	L-Leucine (0.005 M) +	0.48	0		13.205‡	40.8
I	glycine* (0.005 M)	0.43	0		13.525§	41.3
II		0.25	2.976	4.709	14.01	43.3
II		0.11	2.259	6.162	13.92	43.0
I	L-Leucylglycine* +	0.37	0		6.060‡	38.5
I	glycine + L-leucine (0.0025 M of each)	0.41	0		5.603§	35.6
I	L-Leucylglycine +	0.45	0		6.588‡	40.7
I	glycine* + L-leucine (0.0025 M of each)	0.45	0		6.763§	41.8
IIp¶	L-Leucylglycine* (0.005 M)	0.28	2.897	3.797	11.00	35.0
IIp¶	L-Leucine (0.005 M) +	0.33	2.694	5.103	13.75	42.5
IIp¶	glycine* (0.005 M)	0.23	3.412	4.434	15.13	46.7
IIp¶		0.14	2.437	6.217	15.15	46.8

* Indicates presence of N^{15}.

† $\frac{N^{15} \text{ in cell protein}}{N^{15} \text{ of all glycine in medium}} \times 100$. The glycine* contained 32.366 atom per cent excess N^{15}; the N^{15} content of the glycine residue of L-leucylglycine* was 31.432 atom per cent excess.

‡ Trichloroacetic acid precipitate washed 3 times with 6 per cent trichloroacetic acid solution.

§ Trichloroacetic acid precipitate washed 4 times with 6 per cent trichloroacetic acid solution.

¶ Medium contained 5 Oxford units of penicillin per ml.

in the last four lines of Table 2. As was to be expected, penicillin did not alter the duration of the lag period for growth in the presence of leucylglycine, but it did increase, by about 25 hours, the lag period in the presence

of the amino acid mixture. Nevertheless, the utilization of glycine-nitrogen for protein synthesis in the presence of penicillin did not differ from its utilization in the absence of the antibiotic. Although it had been concluded that penicillin interferes with the assimilation of glycine, the data in Table 2 do not indicate any alteration in the rate at which glycine-nitrogen enters into the bacterial proteins once growth has started. It should be emphasized, however, that these data describe the protein nitrogen of cultures only during the final 2 or 3 cell divisions, and that at least 5 cell divisions had occurred prior to the first sampling of the bacteria. Since the action of penicillin on strain SF is to prolong the lag period without altering the generation time, it may well be that an effect of penicillin on the uptake of glycine-nitrogen would be observed only during the first one or two generations.

Effect of glycine on the growth response to L-*leucine.* In an effort to clarify the effect of glycine on the growth response of strain SF to leucine, the organism was grown in media containing 0.001, 0.005, or 0.008 M leucine, and each level of leucine was supplemented with 0.001, 0.003, 0.005, and 0.008 M glycine. The extent of growth observed with these mixtures was determined almost entirely by the leucine concentration. The addition of as much as 0.008 M glycine to 0.001 M leucine increased the maximal turbidity of the culture only slightly above that observed with 0.001 M leucine alone. However, as little as 0.001 M glycine was sufficient to reduce the lag period and to decrease the generation time characteristic of the growth response to leucine. An increase in the glycine concentration to 0.005 M did not reduce the lag period further, but stimulated the initial growth rate appreciably.

The diphasic growth curve obtained with a mixture of 0.008 M leucine + 0.008 M glycine was described in an earlier section. Similar diphasic curves were seen with mixtures of 0.005 M leucine + 0.008 M glycine, and of 0.008 M leucine + 0.005 M glycine. In these curves, the growth rate during the first rise seemed to be a function of the glycine concentration, while the rate during the second rise was always similar to that for the final portion of a growth curve for leucine alone.

The presence of 5 Oxford units of penicillin per ml. of medium produced changes in the lag period similar to those observed earlier with unsupplemented glycine. As before, when the amino acid mixtures contained 0.001 M glycine, penicillin had no apparent effect on the duration of the lag period; the inhibitory action was evident at glycine concentrations of 0.003 M or higher, and the prolongation of the lag period was most pronounced at 0.005 M glycine.

Reversal of penicillin inhibition by L-*leucylglycine.* In order to determine whether leucylglycine can reverse the inhibitory action of penicillin, media containing penicillin and glycine (0.005 M) were supplemented with vary-

ing amounts of the dipeptide (0-0.005 M). The growth curves obtained with these media were compared with the curves for the same mixtures of glycine and leucylglycine in penicillin-free media. A parallel experiment was conducted in which leucine (0.005 M) also was present in the media. Some of the growth curves for the more complex mixtures (glycine + 0.003 or 0.005 M leucylglycine; glycine + leucine + 0.0005-0.005 M leucylglycine) exhibited the diphasic character previously observed with mixtures of glycine + leucine. The optical density at which each diphasic curve showed a change in growth rate appeared to depend upon the concentration of leucylglycine in the medium. Furthermore, the second rise of the curve was small with media containing leucylglycine + glycine, but was appreciable with media containing leucine in addition to the dipeptide and glycine. These observations suggest that, under the conditions of the above experiments, the metabolism of strain SF was directed initially toward the utilization of leucylglycine for growth, and that the metabolism of the free amino acids in the medium became the rate-determining factor only during the latter stages of growth.

In a medium containing penicillin (5 Oxford units per ml.) and glycine (0.005 M), as little as 0.0005 M leucylglycine was sufficient to produce a demonstrable shortening of the lag period observed in the absence of the dipeptide. With higher concentrations of leucylglycine (0.003-0.005 M), penicillin inhibition was completely prevented.

The reversal of penicillin inhibition by leucylglycine may be explained on the basis of the preferential utilization of the peptide for growth, noted above. As shown earlier, the prolongation, by penicillin, of the lag period in glycine-containing media appears to be a result of an inhibition of glycine assimilation by strain SF. When leucine is also present in the medium, the action of the antibiotic causes a concomitant delay in the utilization of this amino acid, as well as glycine. It may be concluded, therefore, that the biochemical processes which determine the duration of the lag period in a medium containing leucylglycine are different from those which determine the initial growth response to the amino acid mixture. The inhibition of glycine utilization by penicillin clearly involves a metabolic reaction important for the initiation of the growth of strain SF in media containing glycine and leucine as the sole organic nutrients. This metabolic pathway appears to be circumvented when glycine is provided in the form of leucylglycine, and the process blocked by penicillin does not seem to be involved in the initial utilization of the dipeptide for growth. It is probable that most of the subsequent metabolic transformations of the amino acid residues of the dipeptide are similar to those characteristic of free glycine and leucine; this is supported by the data, described earlier, dealing with the incorporation of isotopic nitrogen into the bacterial proteins. These latter processes do not appear to be significantly influenced by penicillin.

It may be of interest, in connection with the foregoing speculations, to

mention some preliminary experiments which have been carried out in an attempt to study the action of penicillin during the lag period. In these tests, larger inocula (approximately 10^9 cells) than employed previously were added to 10 ml. of media containing leucylglycine (0.005 M), leucine (0.005 M), or a mixture of leucine (0.005 M) + glycine (0.005 M). Under these conditions, the optical density of the cultures increased from about 0.05 to 0.44, corresponding to approximately 3 cell divisions. In media containing leucylglycine or leucine, logarithmic growth began within 3 hours, both in the presence and in the absence of penicillin; the generation times were the same as those observed with the smaller inoculum employed in earlier experiments. With the large inoculum, the response of the culture in the presence of the amino acid mixture was quite different from that noted with leucylglycine or leucine. Logarithmic growth was initiated only after *ca.* 15 hours; during this time, the turbidity of the culture increased from an optical density of 0.05 to 0.075. A similar small increase in turbidity was observed in the medium containing the amino acid mixture and penicillin (5 Oxford units per ml.). In the presence of penicillin, however, growth appeared to cease after 15 hours, and did not resume until 18 hours later, when the logarithmic rate became evident. Although no viable cell counts were performed during the stationary phase of growth in the presence of the amino acid mixture and penicillin, the optical density of the culture did not decrease, and this may be taken to indicate that the cell population did not diminish significantly.

Behavior of strain SF grown in the presence of penicillin or incubated in basal medium containing penicillin. It seemed of importance to determine whether the growth of strain SF in media containing penicillin is a consequence of the appearance, in the culture, of bacterial cells that are "adapted" to the bacteriostatic action of the antibiotic. Therefore, cells were allowed to grow to the maximal optical density on a mixture of leucine and glycine (0.005 M of each) in the absence and in the presence of penicillin (5 Oxford units per ml.), and then were tested for their sensitivity to penicillin. The growth response of such cells, on transfer to control media containing leucylglycine, the mixture of leucine + glycine, leucine, or glycine, and to a similar series of media supplemented with penicillin, gave no indication of adaptation. The inocula which were taken from the medium containing penicillin were just as sensitive to the antibiotic as the inocula from penicillin-free medium.

In another experiment, cells (10^9 per 10 ml.) from an agar slant were incubated in the basal medium with penicillin (50 Oxford units per ml.) for 5 hours. Cell counts, made at the start of the incubation and after 2.5 and 5 hours, showed no detectable change in the number of viable cells. The growth response of the cells was then tested by inoculation into a series of media similar to that employed in the above experiments on adaptation. Before transfer of the cells into the test media, the incubation culture was

centrifuged, the supernatant solution was decanted, and the cells were resuspended in water. Untreated controls (cells incubated in the basal medium devoid of penicillin) responded to the amino acid mixture and to glycine in the usual manner, and, in the presence of penicillin in the test medium, exhibited the expected prolongation of the lag periods. The growth response of the penicillin-treated cells to the amino acid mixture and to glycine in test media to which penicillin had not been added was identical with the growth response of the untreated controls and of penicillin-treated cells in penicillin-containing test media. Thus, incubation of strain SF in the basal medium containing penicillin led to an alteration in the metabolic behavior of the organism. This alteration appears to be

TABLE 3

A REPRESENTATIVE EXPERIMENT SHOWING THE EFFECT OF SUPPLEMENTARY CARBON COMPOUNDS

All media contained glycine (0.005 M). Penicillin was used at a level of 5 Oxford units per ml. of medium.

Carbon compound		*Lag period*		*Generation time**	*Final optical density**
	Concentration of carbon	*Penicillin absent*	*Penicillin present*		
	moles per liter	*hours*	*hours*	*hours*	
None		30	49	8	0.070
Glucose	0.0003	18	36	10	0.076
	0.003	11	13	7	0.11
Glycerol	0.0003	24	44	9	0.070
	0.003	20	26	7	0.11
Sodium acetate	0.0003	20	38	10	0.070
	0.003	10	13	6	0.10

* The generation time and the final optical density are not altered by the presence of penicillin in the medium.

concerned with the ability of the cells to assimilate free glycine, for the penicillin-treated cells gave completely normal growth in media containing leucylglycine or leucine, but no glycine.

Reversal of penicillin inhibition by non-nitrogeneous compounds. It was of interest to find that in the presence of glucose and of other non-nitrogenous carbon sources (glycerol, acetate) in the medium, the inhibitory action of penicillin on glycine assimilation was counteracted (Table 3). The non-nitrogenous compounds were used in relatively low concentrations so that the extent of growth in their presence would not be much greater than that observed with unsupplemented glycine (0.005 M). When the concentration of supplementary carbon was 0.0003 M, the presence in the medium of glucose, glycerol, or acetate decreased the lag period, but had

no effect on the extent of inhibition by penicillin. At a higher concentration (0.003 M) of supplementary carbon, however, the lag period was greatly reduced, and the effect of penicillin was almost completely overcome. Glycerol was less effective in this regard than were glucose or acetate.

From these data it would appear that, when a sufficient quantity of a readily utilized carbon compound is present in the medium, glycine assimilation is not inhibited by penicillin. As will be shown in a later section of this communication, strain SF, in the presence of glucose, utilizes glycine primarily as a source of ammonia-nitrogen; earlier data have already indicated that the utilization of ammonia for protein synthesis is not inhibited by penicillin.

TABLE 4

A REPRESENTATIVE EXPERIMENT SHOWING THE EFFECT OF ALTERATIONS IN THE BASAL MEDIUM

The concentration of the dipeptide and of each amino acid was 0.005 M. Penicillin was used at a level of 5 Oxford units per ml. of medium. T_{lag} denotes the lag period, and T_G, the generation time.

Salts in medium (grams per liter)			*Growth response*									
			L-*Leucylglycine*		L-*Leucine + glycine*			L-*Leucine*		*Glycine*		
					T_{lag}					T_{lag}		
NaCl	K_2HPO_4	KH_2PO_4	T_{lag}*	T_G†	*Pen. absent*	*Pen. present*	T_G†	T_{lag}*	T_G†	*Pen. absent*	*Pen. present*	T_G†
			hrs.	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*	*hrs.*
5.0	2.0	1.0	8	9	16	26	9	20	22	28	42	9
2.5	2.0	1.0	9	6	16	19	8	22	20	28	32	8
1.25	2.0	1.0	9	5	15	14	7	20	16	26	28	6
0	2.0	1.0	8	5	15	14	5	20	16	27	27	5
5.0	0.2	0.1	8	9	15	16	8	20	16	27	27	8

* The presence of penicillin in the medium does not affect the lag period in media containing leucylglycine or leucine.

† The generation time is the same in the presence and in the absence of penicillin.

Effect of changes in the basal medium on penicillin inhibition. Experiments in which the concentrations of sodium chloride and of phosphate in the basal medium were varied indicate that the inhibitory effect of penicillin on the growth of strain SF is influenced by the inorganic ions in the medium (Table 4). In media in which the concentrations of either the sodium chloride or of the phosphate were decreased, penicillin (5 Oxford units per ml.) had little or no inhibitory effect on the growth response to glycine (0.005 M) or to the mixture of leucine (0.005 M) + glycine (0.005 M). It is of interest that variations in the inorganic salts of the medium significantly alter the generation time of strain SF, but do not influence

markedly the duration on the lag period. Further experiments are required to elucidate the role of the inorganic ions of the basal medium in the response of strain SF to penicillin.

Utilization of various amino acids for growth. In Table 5 are given the results of experiments in which a number of amino acids were tested for

TABLE 5

A REPRESENTATIVE EXPERIMENT SHOWING THE GROWTH RESPONSE TO AMINO ACIDS

The amino acids were the sole organic nutrients added to the basal medium. Unless otherwise noted, the concentration of each amino acid was 0.005 M. The first turbidity measurement was made 14 hours after inoculation.

Amino acid	*Lag period*	*Generation time*	*Final optical density*
	hours	*hours*	
Glycine	30	8	0.079
L-Alanine	14	5	0.18
L-Serine	35	22	0.16
L-Cystine			0 at 182 hours
DL-Threonine	>140		0.041 at 182 hours
L-Asparagine	<14	5	0.20
L-Valine	<14	5	0.33
L-Glutamic acid	<14	5; 10*	0.26
L-Proline	<14	6	0.35
L-Methionine	*ca.* 140		0.020 at 182 hours
L-Leucine	20	24	0.46
L-Isoleucine	90	*ca.* 40	0.47
L-Lysine·HCl			0 at 182 hours
L-Histidine·HCl	<14	6	0.33
L-Arginine·HCl			0 at 182 hours
L-Phenylalanine	25	10	0.45
L-Tyrosine (0.002 M)†	<14	7	0.25
L-Tryptophan	<14	9	*ca.* 0.45‡
L-Leucine + glycine	15	8	0.46
L-Serine + glycine	15	8	0.22
L-Isoleucine + glycine	30	8	0.45

* Growth curve diphasic. The change in slope occurred at a density of about 0.15.

† At a concentration of 0.005 M, tyrosine precipitated out of the medium after it has been autoclaved. Growth under these conditions is accompanied by the development of a red color in the medium.

‡ A red color appears in the medium during the latter part of the growth period.

their ability to serve as the sole sources of carbon and nitrogen for the growth of strain SF. Of the amino acids tested, L-cystine, L-lysine, and L-arginine showed no growth-promoting action whatever, and DL-threonine and L-methionine were only slightly effective. As will be seen from Table 5, there was considerable variation in the duration of the lag period and in the generation time, depending on the growth-promoting amino acid present;

the extent of growth was approximately proportional to the carbon content of the medium. Penicillin (5 Oxford units per ml.) had no detectable effect on the growth response to any amino acid except glycine.

It was noted earlier that the growth response of strain SF to leucine was characterized by a prolonged lag period and generation time. Serine and isoleucine caused a similar growth response. Furthermore, the response of the organism to equimolar mixtures of serine + glycine or of isoleucine + glycine resembled that observed with a mixture of leucine + glycine (Table 5). The inhibitory action of penicillin in media containing the

TABLE 6

A REPRESENTATIVE EXPERIMENT SHOWING THE GROWTH RESPONSE TO PEPTIDES

Peptide	*Concentration*	*Lag period*	*Generation time*	*Final optical density*
	moles per liter	*hours*	*hours*	
L-Leucylglycine	0.0025	8*	8	0.25
L-Leucylglycine	0.005	8*	8	0.44
L-Leucylglycylglycine	0.005	*ca.* 15†	‡	0.49
L-Leucyl-L-tyrosine	0.005	15*	10	0.57
Glycyl-L-leucine	0.0025	19*	12	0.23
Glycyl-L-leucine	0.005	19*	12	0.47
Glycylglycine	0.005	25*	17	0.12
Glycyl-D-leucine	0.0025	No visible growth after incubation for 264 hours.		
Glycyl-L-leucylglycine	0.0025			
Glycyl-D-leucylglycine	0.0025			
Glycyl-L-leucyl-L-leucine	0.0025			
Glycyl-L-leucyl-D-leucine	0.0025			
Glycylglycyl-L-leucylglycine	0.0025			

* Five Oxford units of penicillin per ml. of medium had no inhibitory effect.
† Penicillin effect has not been tested.
‡ Generation time not determined.

mixture of leucine + glycine was evident in media containing the mixture of serine + glycine or of isoleucine + glycine. These observations suggest that, in the assimilation of the three amino acid mixtures investigated thus far, there is a similar metabolic process which is inhibited by penicillin.

Although penicillin inhibited growth in media containing glycine (0.005 M) supplemented with carbon (0.015 M) supplied as serine, no inhibition was noted when supplementary carbon (0.003 M) was supplied as glucose, glycerol, or acetate (cf. Table 3). In the absence of penicillin, the growth responses in both types of media were similar, as might have been expected

from the known metabolic relationship of serine to intermediates in carbohydrate metabolism, via pyruvic acid. The difference in the penicillin effect, however, provides additional evidence for the view that the antibiotic blocks the assimilation of amino acids.

Utilization of various peptides for growth. A number of peptides of leucine and of glycine were tested for their ability to serve as sources of nitrogen and of carbon in the growth of strain SF (Table 6). Glycyl-L-leucylglycine, glycyl-L-leucyl-L-leucine, and glycylglycyl-L-leucylglycine did not promote bacterial growth; this indicates that, under the experimental conditions employed, the organism is unable to obtain leucine or glycine from these peptides. This is in contrast to the behavior of a *leucineless* mutant of *Escherichia coli* which can readily use all three compounds to satisfy its requirement for an exogenous source of leucine.[12]

Strain SF was able to utilize glycyl-L-leucine for growth; the growth curves at concentrations of 0.0025 to 0.02 M were identical except for the maxima, which increased with increasing concentrations of the dipeptide. The response to glycyl-L-leucine was characterized by a longer lag period and a longer generation time than those noted with L-leucylglycine or an equivalent mixture of leucine + glycine. However, penicillin did not inhibit growth on glycylleucine, and, in this respect, the growth response to glycylleucine differs profoundly from the response to the mixture of leucine + glycine.

The dipeptide L-leucyl-L-tyrosine also was utilized by strain SF for growth. With 0.002 M leucyltyrosine, the lag period was 15 hours and the generation time was 10 hours. On the other hand, growth on a mixture of tyrosine (0.002 M) + leucine (0.002 M) was characterized by a shorter lag period (less than 14 hours) and a shorter generation time (7 hours). It would appear, therefore, that the less effective utilization of the dipeptide may be a consequence of the fact that enzymatic hydrolysis of the compound is the initial step in its metabolism. A similar conclusion may be offered in the case of the utilization of glycylleucine by strain SF.

Glycylglycine was found to promote the growth of strain SF at all concentrations tested (0.0025-0.04 M). The extent of growth increased as the concentration of the dipeptide was raised, but the lag periods were approximately the same (about 25 hours). This may be contrasted with the increasing lag period (30 to 90 hours) observed with glycine at concentrations of 0.005 to 0.02 M, and with the absence of growth at a glycine concentration of 0.04 M. The generation time in the presence of glycylglycine (17 hours) was about twice as long as that for glycine, which suggests that glycylglycine, like glycyl-L-leucine and L-leucyl-L-tyrosine, may be hydrolyzed as the initial step in its utilization for growth.

With concentrations of glycylglycine up to 0.01 M, the extent of growth was approximately the same as that produced by equivalent concentrations

of glycine. However, penicillin (5 Oxford units per ml.) did not inhibit the growth response to any concentration of the dipeptide employed in these experiments. It will be recalled that penicillin had a marked effect in media containing glycine at a concentration of 0.03 M or higher. Thus, the results of tests with glycylglycine offer additional support for the view that the assimilation of glycine *per se* differs from the assimilation of glycine supplied to strain SF in the form of glycine peptides.

Growth of strain SF in media containing glucose

Growth response to L-*leucylglycine,* L-*leucine, and glycine.* In Table 7 are presented the data obtained in an experiment in which leucylglycine (0.005 M), leucine (0.005 M), glycine (0.005 M), or a mixture of leucine (0.005 M) + glycine (0.005 M) was added to the basal medium supplemented with glucose (0.017 M). The growth response was the same in media containing leucylglycine, glycine, or the amino acid mixture, but with leucine the lag period and generation time were longer and the extent of growth was much less. The limited growth with leucine may be due to the fact that the final pH of the medium was 3.5 while, with the other test compounds, the final pH was never lower than 6.5. Cultures of strain SF in a medium containing glucose and leucine developed an odor characteristic of fatty acids. This observation merits further study.

TABLE 7

A REPRESENTATIVE EXPERIMENT SHOWING THE GROWTH RESPONSE OF STRAIN SF IN MEDIA CONTAINING GLUCOSE

Each nitrogenous compound was present in a concentration of 0.005 M. The concentration of glucose was 0.017 M.

Test compound	*Lag period*	*Generation time*	*Final optical density*
	hours	*hours*	
L-Leucylglycine	4.5	8	1.19
L-Leucine + glycine	4.5	8	1.21
L-Leucine	*ca.* 18	*ca.* 30	0.51
Glycine	4.5	7	1.06

The maximal growth observed with leucylglycine as the nitrogen source corresponded to an optical density of about 1.1, and was produced by a peptide concentration of 0.003 M and higher. Glycine (0.006 M) or a mixture of 0.003 M glycine + 0.003 M leucine gave the same optical density. In the glucose-containing medium, the extent of growth in the presence of ammonium chloride was the same as that produced by equivalent concentrations of the organic nitrogen compounds. These results suggest that,

under these conditions, the test compounds were merely serving as nitrogen sources. The inhibitory action of high concentrations of glycine, observed previously in glucose-free media, also was evident in media containing glucose. At glycine concentrations of 0.04 M or higher, the organism did not grow, and, at a concentration of 0.03 M, growth occurred only after a prolonged lag period.

Action of penicillin. In the glucose-containing medium, penicillin (5 Oxford units per ml.) had no bacteriostatic action on strain SF when glycine (0.005 M) served as the nitrogen source. This was also true at a much higher penicillin concentration (300 Oxford units per ml.); at 1000 Oxford units per ml., however, some toxic action became apparent. At the latter level, the lag period in the presence of leucylglycine or of leucine was prolonged about 9 hours; this was probably due to the bactericidal action of the antibiotic, since cell counts made 5 hours after inoculation showed that only 5-10 per cent of the cells of the original inoculum were viable. In the presence of glycine or of the mixture of leucine + glycine, the lag period was prolonged about 30 hours when the medium contained 1000 Oxford units of penicillin per ml. The viable cell count, made 5 hours after inoculation, showed the same "kill" of 90-95 per cent found for media containing leucylglycine or leucine. The prolonged lag periods may have been due to further destruction of the viable cells remaining in the media after the 5-hour sampling, or to the bacteriostatic action of penicillin observed in glucose-free media, or to a combination of the two effects. In any event, strain SF appeared to be much more sensitive to the action of penicillin in media containing free glycine than in media containing a peptide of glycine.

Utilization of glycine-nitrogen for protein synthesis. Since the data obtained for the growth response of strain SF to leucylglycine and to the mixture of leucine + glycine in the medium containing glucose suggested that, under these conditions, the peptide and amino acids served merely as sources of nitrogen, it seemed desirable to investigate the relative extent to which the nitrogen of glycine or of the glycine residue of the peptide is utilized for protein synthesis. Isotopic glycine and isotopic leucylglycine, therefore, were supplied to the organism in a glucose-containing medium, and the N^{15} content of the bacterial proteins was determined after the culture had reached maximal growth. These tests were conducted in parallel to Experiment I cited in Table 2; the procedure was the same as that described previously.*

A comparison of the data presented in Table 8 with those given in Table 2 shows that, under comparable conditions, glycine-nitrogen is utilized

* The authors are greatly indebted to Dr. Henry D. Hoberman for the isotopic glycine used in these experiments. They wish also to acknowledge the generous coöperation of Dr. Hoberman and Mr. Joseph Doolittle in the conduct of the isotope experiments.

more effectively for protein synthesis in glucose-containing media. It is of interest that no appreciable difference was noted in the extent of N^{15} incorporation from isotopic glycine and from an equivalent amount of isotopic leucylglycine. However, the N^{15} was incorporated into the bacterial proteins to a greater extent when the glycine concentration of the medium was 0.005 M than at a level of 0.0025 M glycine. These results support the

TABLE 8

N^{15} EXPERIMENTS WITH STRAIN SF GROWN IN MEDIA CONTAINING GLUCOSE
The glucose concentration was 0.01 M.

*Test compound**	*Concentration*	*Optical density of culture*	*N^{15} in cell protein*	*Protein-N derived from glycine-N of media†*
	moles per liter		*atom % excess*	*%*
L-Leucylglycine*	0.0025	0.92	15.896‡	50.6
	0.0025	0.92	15.956§	50.8
	0.005	0.84	17.405‡	55.4
	0.005	0.85	18.406§	58.6
L-Leucine + glycine*	0.0025 of each	0.99	16.316‡	50.4
		1.00	16.305§	50.4
	0.005 of each	0.97	17.595‡	54.4
		0.96	17.555§	54.3
L-Leucylglycine* + glycine + L-leucine	0.0025 of each	0.85	8.510‡	54.1
		0.87	8.965§	57.0
L-Leucylglycine + glycine* + L-leucine	0.0025 of each	0.92	9.325‡	57.6
		0.93	9.345§	57.6

* Indicates presence of N^{15}.

† $\frac{N^{15} \text{ in cell protein}}{N^{15} \text{ of all glycine in medium}} \times 100$. The glycine* contained 32.366 atom per cent excess N^{15}, and the glycine residue of L-leucylglycine* 31.432 atom per cent excess.

‡ Trichloroacetic acid precipitate washed 3 times with 6 per cent trichloroacetic acid solution.

§ Trichloroacetic acid precipitate washed 4 times with 6 per cent trichloroacetic acid solution.

view that, in the presence of glucose, strain SF utilizes glycine and leucylglycine principally as sources of nitrogen. This is in contrast to the response of the organism in glucose-free media, where a marked difference was noted in the utilization of free glycine and of peptide glycine, thus illustrating further the striking variations in the nitrogen metabolism of strain SF when the sources of carbon are altered.

Summary

The growth of strain SF, a Gram-negative bacillus, to the dipeptide L-leucylglycine is characterized by a shorter lag period than that observed with an equivalent mixture of L-leucine and glycine, when these test substances serve as the sole carbon and nitrogen sources for the organism. Strain SF can utilize a number of amino acids as the sole sources of carbon and nitrogen, but of those which have been tested, L-cystine, L-lysine, L-arginine, L-methionine, and DL-threonine were ineffective in promoting bacterial growth. The growth response to glycine, but not to the other amino acids tested, is inhibited by penicillin. When glycine is supplied in the form of the peptides L-leucylglycine, glycyl-L-leucine, or glycylglycine, the bacteriostatic action of penicillin is not evident.

Penicillin inhibition of growth in a medium containing glycine is prevented by the addition of L-leucylglycine but not by the addition of L-leucine, L-isoleucine, or L-serine. Relatively small amounts of glucose, glycerol, or acetate also reverse the bacteriostatic action of penicillin. The bacteriostatic action of the antibiotic can also be altered by changes in the concentrations of the inorganic salts in the basal medium. The bactericidal action of high concentrations of penicillin is more pronounced in media containing glucose and glycine than in media containing glucose and L-leucylglycine or L-leucine.

Experiments with glycine and with L-leucylglycine containing N^{15} in the glycine-nitrogen show that the nitrogen of the free amino acid is used more effectively for protein synthesis than is the nitrogen of peptide glycine only if the cells are grown in the absence of glucose. However, more of the nitrogen of both free and peptide glycine is incorporated into the bacterial proteins in the presence of glucose than in the absence of the carbohydrate.

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NEWS AND COMMENT

Excerpt from the Minutes of the Board of Permanent Officers

George Milton Smith (July 5, 1879—February 26, 1951). Working quietly, calmly, and at times, it seemed, almost obscurely, Dr. George Milton Smith acquired international distinction in his major field of interest. He worked obscurely in that he never sought renown; he won distinction for his role in marshalling the attack on the unknowns of cancer. Cancer was no recent interest of his when he became a member of the Cancer Unit and, later, Chairman of the Atypical Growth Research Unit at Yale. He had already served briefly as Director of the Barnard Free Skin and Cancer Hospital before World War I, in which he was Captain in Mobile Hospital No. 39.

Upon assuming new responsibilities at Yale, he familiarized himself with programs of cancer research throughout the world. This background enabled him effectively to stimulate further research. As Medical Director, he participated in the program of the Anna Fuller Fund from its inception and was a member of the Board of Scientific Advisers of The Jane Coffin Childs Memorial Fund. He was also a Director of the American Society for the Control of Cancer at the time of its reorganization as the American Cancer Society. A member of the first National Advisory Cancer Council of the U. S. Public Health Service, Dr. Smith served as Executive Director of the Council from 1944-1947—the period of greatest expansion in its program.

Few researches on abnormal growth escaped his consideration, and every deserving investigator, at Yale or elsewhere, who sought his counsel or aid was rewarded. His broad understanding of the cancer problem, his personal kindness and charm, and his keen judgment contributed to the rapid development of programs and support of cancer research. Despite a full schedule, he still found time for his own investigations on cancer in fishes and reptiles.

Dr. Smith's intimate contact with the problems of industrial health, as the result of his years of experience as surgeon to the Scovill and American Brass Companies, made him a natural leader during World War II in the programs designed to maintain health under the rigorous conditions of rapid industrial expansion. He was a member of two national committees on industrial health and welfare, and Chairman of the Subcommittee on Armored Vehicles of the National Research Council. His interest and experience also enabled him to aid in the establishment at Yale of the Institute of Industrial Health and Medicine, now integrated with the School of Public Health.

Finally, Dr. Smith was an anatomist with a keen interest in applied and functional anatomy, in comparative morphology, as well as in the history of anatomy. He provided for the once bare walls of the corridor in the Department of Anatomy a fine collection of prints portraying the early anatomists, and thus he shared with others his instincts as a collector and his familiarity with our predecessors who have contributed to our knowledge of the body's structure. His collection of old and rare volumes on

ichthyology is a monument to his scholarly interest in the development of comparative morphology and marine life.

As a scholar, as a surgeon interested in man's health, and as a leader in the development of cancer programs, Dr. Smith has enriched our heritage in thought, in accomplishment, and in scholarly collections and studies. His devotion and loyalty to Yale and its School of Medicine, his graciousness, kindness, and generosity were exemplary. Although he shall no longer work among us, his pervasive spirit will long be with us.

WILLIAM U. GARDNER

EVENTS

PHARMACOLOGY SEMINAR

February 1, 1951

SOME THINGS THAT ISOTOPES HAVE TAUGHT US ABOUT DRUG ACTION. By Donalee L. Tabern, Head of Special Research, Abbott Laboratories, North Chicago, Illinois.

Radioactive isotopes are used as diagnostic and therapeutic tools and in studies of drug distribution and metabolism. In tagged drugs so far studied no correlation between distribution and site of action has been found, although much information has been gained with regard to metabolism of drugs and to thyroid physiology. In diagnostic procedures, iodinated serum albumin has been used to measure blood volume; iodine labelled serum globulin showed the liver and lung to be the site of antibody-antigen formation; tagged fluorescein dyes are useful in detecting brain tumors; and the uptake of radio-iodine is a more reliable index of thyroid dysfunction than measurement of the basal metabolic rate. In addition to the treatment of thyroid diseases with radioactive iodine, recent application of colloidal radio-gold in the treatment of malignant ovarian tumors and lymphosarcomas has yielded encouraging results.

J. P. G.

PHARMACOLOGY SEMINAR

February 8, 1951

ION EXCHANGE RESINS AS BIOCHEMICAL AND PHARMACOLOGICAL RESEARCH TOOLS. By Arthur Deming, Head of Biochemical Research, Smith, Kline, and French, Inc., Philadelphia, Pennsylvania.

Cation exchange resins contain either carboxyl or sulfonate groups which have the capacity of releasing hydrogen ions in exchange for metallic ions; anion exchange resins usually are tertiary or quaternary nitrogen compounds and will bind anions. Of high molecular weight and great insolubility, both have been used in technological processes, such as the purification of water and fractionation of blood, and more recently in biological research and therapy. To remove sodium from patients in cardiac edema, cation exchange resins have been administered orally, usually with potassium incorporated in the resin to prevent acidosis and hypokalemia. The resin appears to potentiate the action of mercurial diuretics and to sensitize patients previously resistant to those drugs. In anuric patients, cation exchange resins have been administered rectally to prevent potassium intoxication. They are also useful in inducing salt depletion in experimental animals. In the treatment of peptic ulcers, anion exchange resins have been tried as antacids. No chloride is removed from the animal, for the alkalinity in the lower portion of the gastro-intestinal tract releases the previously bound chloride which is then reabsorbed.

J. P. G.

YALE MEDICAL SOCIETY

February 14, 1951

INFECTION OF CHIMPANZEES WITH COXSACKIE VIRUSES. By Albert S. Kaplan, Lisbeth M. Kraft, and Joseph L. Melnick.

Chimpanzees fed the Coxsackie group of viruses developed an infection which was studied by the titration of virus isolated from the throat,

stools, and blood, and by following the development of neutralizing and complement-fixing antibodies in the sera. Each of the four animals studied became throat and stool carriers when first fed virus; when challenged with an homologous strain, virus was excreted in the stools for a short time, which is interpreted as being a passive transfer of virus through the intestinal tract. Virus was never isolated from the throat after homologous challenge. When these animals were fed an heterologous strain of virus, however, they were again infected, as indicated by the appearance of virus in both the throat and the stools and by the development of neutralizing antibodies specific for the strain fed. These animals exhibited immunity to homologous strains for at least one year. The results of the complement-fixation tests were quite different, however, in that each animal manifested a response peculiar to itself. Although rises in homologous antibody titer always occurred after the feeding of different viruses, there were many occasions when antibodies to heterologous viruses rose at the same time whether the animal had previously been fed these latter types or not. It was suggested that these represented anamnestic responses or that the Coxsackie viruses in primates are able to display by the complement fixation tests some relationship that is not revealed by the neutralization antibody tests in lower animals or in any animal.

A. S. KAPLAN

EFFECTS OF EXERCISE, EMOTIONAL STRESS, AND ADRENALIN ON THE RENAL EXCRETION OF SALT AND WATER. By William D. Blake.

The effects of exercise, emotional stress, and adrenalin were studied on unanesthetized, trained, female dogs, using standard techniques for measuring glomerular filtration rate, renal plasma flow, filtration fraction, and sodium and water excretion. Treadmill exercise alone caused no significant change in any of the functions. Emotional stimulation, elicited by sounding a bicycle klaxon, resulted in a decrease in sodium excretion without significant change in any other urinary functions. Finally, adrenalin administered with a simultaneous sodium salt infusion induced a decrease in sodium excretion. This decrease was more apparent in non-hydrated dogs and less apparent in dogs made hypervolemic with hypertonic sodium solution. In the latter animals the decrease was related to parallel changes in filtration rate.

Since none of the changes in sodium excretion was associated with changes in glomerular filtration rate (with the exception mentioned), the influence of adrenalin and emotion was presumed to be exerted upon tubular function.

J. F. S.

ELECTRON MICROSCOPE STUDIES OF HUMAN PAPILLOMAS CHARACTERIZED BY INTRANUCLEAR INCLUSION BODIES. By M. J. Strauss, H. Bunting, and J. L. Melnick.

A virus etiology of verrucal lesions has been presumed for many years. The present investigation was designed to clarify certain clinical and pathological findings. A clinically distinct type of wart served as the experimental material—a wart appearing different from the usual verruca vulgaris, having a more pearly base and an erythematous halo, and being readily separated from the underlying tissue. Particles were demonstrated with ease by centrifugation and electron microscopy from extracts of warts having this appearance but not from other warts. These uniform and

spherical particles were distinguished by their crystalline-like clustering, their diameter being of the same order as that of the Shope rabbit papilloma.

Histological examination of the papillomas revealed further correlation between the presence of the particles and anatomical manifestations. Many of the cells of the Malpighian layer contained intranuclear inclusion bodies and cytoplasmic masses. The affected cells were abnormal in that they lacked intercellular bridges, were never observed in mitosis, had abnormalities of nuclear structure, contained no keratohyaline granules and showed parakeratosis in the stratum corneum. Additional cases presenting this histological picture were found in a survey of surgical material. It was suggested, then, that these crystalline particles interfere with the normal process of growth and differentiation in this type of wart.

O. L. K.

PHARMACOLOGY SEMINAR

February 15, 1951

CONTROL OF CARDIAC ARRHYTHMIAS WITH PROCAINE AND OTHER DRUGS. By E. A. Rovenstine, Professor of Anesthesiology, New York University-Bellevue Medical Center, New York, New York.

Procaineamide and procaine have been used extensively to control the cardiac arrhythmias which frequently occur in cyclopropane anesthesia. Procaineamide, in contrast to procaine, is not destroyed by plasma procaine-esterase, it is slowly metabolized, and it is totally absorbed after oral administration. The convulsant properties of procaine are absent in the amide. Topical application of procaine to the heart is more potent in reversing ventricular tachycardia than intravenous administration of either procaine or procaineamide. Evidence is accumulating that these agents can control arrhythmias in unanesthetized patients.

J. P. G.

CARDIOVASCULAR STUDY UNIT

February 20, 1951

THE MERCURIAL DIURETICS IN CONGESTIVE HEART FAILURE. By Arthur C. DeGraff, M.D., New York University College of Medicine, New York, New York.

The aims of therapy in congestive heart failure can be considered twofold: to make the circulation adequate, and to prevent edema when the circulation remains inadequate. It is in the latter instance that the rationale for the use of mercurial diuretics is found. To this end the diuretics are employed either alone or in conjunction with mechanical removal of the fluid and/or restriction of the sodium intake.

Experiments have shown that when injected intraperitoneally into the mouse, the lethal dose of Thiomerin is much larger than that of Mercuhydrin or Mercuzanthin. It has also been demonstrated in dogs that there is an optimum dose for the mercurial diuretics; any excess over this dose produces very slight if any increase in urine output and makes the danger of ventricular fibrillation more imminent.

The most effective method of administering the mercurial diuretics is via the intravenous route; intramuscular injections are slightly less effective. Thiomerin is the one drug which can be given subcutaneously with good results. Oral administration of these drugs has limited usefulness,

being employed mainly to maintain a patient over long periods of time when symptoms are not severe. Experience suggests that mercury is stored somewhere in the body when mercurials are given orally for extended periods of time; mercury poisoning may result.

The mercurials presumably produce diuresis by reducing tubular reabsorption of water and sodium, but urinary excretion of chloride approximates that of sodium. After about one hour the mercurial diuretic may also be found in the urine. If urinary output is impaired, the drug may accumulate in the body. Ammonium chloride seems to enhance the action of the mercurial diuretics by about 20 per cent. It has not been established, however, whether the ammonium chloride is most advantageous when administered continuously or intermittently.

In patients previously responding to mercurial diuretics, these drugs may lose their effect if the patient is given morphine or demerol. This phenomenon may be due to stimulation of secretion of antidiuretic hormone by the narcotic agents mentioned. One patient receiving barbiturates has also shown diminished response to mercurial compounds which were previously effective, although most patients show no reduction in response to them when given barbiturates.

It has long been thought that among the toxic effects resulting from the use of mercurial diuretics, digitalis toxicity was due to the mobilization of digitalis from the extravescular fluid compartment by the diuretic. Recent experiments suggest, however, that depletion of potassium from cells, which may occur in diuresis, increases cell sensitivity to digitalis and, therefore, increases digitalis effect. Patients receiving digitalis and a mercurial diuretic who have developed ventricular tachycardia have been given potassium infusions and continued on their usual dose of digitalis, yet the tachycardia has disappeared.

K. G. B.

PLANT SCIENCE SEMINAR

February 23, 1951

CYTOPLASMIC STERILITY. By Herbert L. Everett, Agricultural Research Assistant, Connecticut Agricultural Experimental Station, New Haven, Connecticut.

From genetic studies of various organisms, including corn, broad bean, mosses, paramecia, tobacco, and sugar beets, evidence has accumulated indicating that cytoplasm is responsible for the inheritance of certain characters. The egg, having a larger quantity of cytoplasm than the sperm, largely determines the expression of the character.

There are several strains of corn which possess the character known as "pollen sterility" or "male sterility." In a cross with "normal" corn, this character is transmitted only when it is carried by the egg. Genetic studies of crosses involving such "male sterile" strains have led to the following hypothesis concerning the origin and transmission of this character: A suitable cross puts a gene into cytoplasmic surroundings different from the "ordinary" ones; under these circumstances a macromolecule of unusual properties is created. This macromolecule is able to duplicate itself and to compete with other protoplasmic factors. No apparent damage is done by this competition in the large cytoplasmic masses of somatic or egg cells, but in the small amount of cytoplasm of the pollen, the effect may be lethal.

There is evidence, from experiments as yet incomplete using crosses between the variety "Minnesota 71" and certain male sterile strains, that

the lethal effect of the macromolecule or particle may be masked. This is indicated by the fact that pollen from some such crosses is sensitive to environment—sterile under poor growing conditions, but fertile under good ones. That the particle has not been destroyed is shown by the appearance of its effect in subsequent crosses.

The nature of the particle is unknown, but it may be virus-like.

Male sterility is of practical importance in the production of some field crops. In those cases in which pollenation must be prevented, as in the production of hybrid seed crops or of tobacco leaves, the pollen sacs must otherwise be removed manually, increasing the cost of production.

R. R. L. G.

PHARMACOLOGY SEMINAR

March 1, 1951

PAIN AND ITS CONTROL. By Henry K. Beecher, Professor of Anesthesiology, Harvard Medical School, Boston, Massachusetts.

Laboratory methods used to investigate pain in animals are not applicable to humans, for there appears to be a difference between experimental and pathological pain. In controlled clinical studies, placebos relieved pain in 20 per cent of patients, pentobarbital in 40 per cent, and morphine in 70 per cent. Subjects given pentobarbital, which is generally believed to be without analgetic action, behaved like patients who were subjected to prefrontal lobotomies; pain was felt but it was not bothersome. Barbiturates are known to block after-discharge, and it was postulated that pain perception requires functional and intact association pathways. A psychic factor is further indicated by the observation that suggestion and stress can block pain. Of the synthetic analgetics tested, racemic methadon and, more particularly, *l*-isomethadon appear most promising. However, the ease with which they can be synthesized from readily available materials also poses a problem in the control of drug addiction.

J. P. G.

PHARMACOLOGY SEMINAR

March 8, 1951

SYMPATHOMIMETIC AMINES: NEWER DEVELOPMENTS. By Alonzo M. Lands, Head of Pharmacology, Sterling-Winthrop Research Institute, Rensselaer, New York.

It is probable that epinephrine and nor-epinephrine, both of which are obtainable from adrenergically innervated organs, differ quantitatively. The depressor action on blood pressure elicited by these substances is followed by an elevated blood pressure. Nor-epinephrine, however, has a greater pressor action and a lesser depressor effect than epinephrine; thus, nor-epinephrine constricts vessels in almost all areas, while epinephrine constricts only some vessels, notably in the cutaneous regions, and dilates others, especially vessels serving skeletal muscle. With increased dosage of either drug, vasoconstriction is dominant. A similar diphasic action of the two substances is observed on the guinea pig's ileum; inhibition follows stimulation in small doses, but in large doses only inhibition is demonstrated. The excitatory and inhibitory responses may possibly be explained on the basis of two different cellular receptors: union of the agent with one receptor results in contraction, and union with the other receptor fosters relaxation. One type of receptor probably predominates in each organ, and

it is this receptor which regulates the response. Since all sympathomimetic amines stimulate the heart, it would follow that the heart contains receptors only for contraction.

Both epinephrine and nor-epinephrine are released upon stimulation of a sympathetic nerve. Initial stimulation results in a preponderance of epinephrine secretion while prolonged stimulation results in an increased release of nor-epinephrine. Physiologically, epinephrine appears to act more like a vasodilator, and nor-epinephrine like a vasoconstrictor. Nor-epinephrine is probably a biological precursor of epinephrine. Although neither of these substances is purely excitatory, a synthetic homologue of epinephrine, isuprel, is purely inhibitory on all organs except the heart.

J. P. G.

PHARMACOLOGY SEMINAR

March 15, 1951

STUDIES IN VASODILATATION. By John C. Krantz, Jr., Professor of Pharmacology, University of Maryland School of Medicine, Baltimore, Maryland.

Investigations of a number of coronary dilators in the nitrate and nitrite series revealed that physiologic activity strikingly paralleled their oil-water partition coefficients. Conferring water solubility on a molecule, such as by introducing a carboxl group, resulted in a loss of dilatory activity. Active nitrates in therapeutic concentrations inhibited ATP-ase of striated and uterine muscles. Oxygen uptake of blood vessels was not affected by these substances. In contrast, sodium nitrite, also an active vasodilator, exhibited no action on ATP-ase but interferred with the oxygen uptake of the arterial muscular wall.

J. P. G.

NU SIGMA NU ANNUAL LECTURE

March 19, 1951

RENAL TUBULAR SECRETION OF INORGANIC IONS. By Alfred Gilman, Professor of Pharmacology, College of Physicians and Surgeons, Columbia University, New York.

Tubular reabsorption of inorganic ions in the kidney is known to represent a process of active transfer, not merely simple diffusion. The mechanism has generally been considered to operate in one direction only —from the tubular lumina to the bloodstream. Experiments suggest, however, that active transfer of potassium occurs in both directions.

Following a potassium infusion in dogs, urine potassium concentration is increased. Further, the potassium/creatinine ratio in the bladder urine is significantly greater than that in the glomerular filtrate, while a mercurial diuretic given at this point produces a sharp fall in rate of potassium excretion. Potassium/creatinine clearance ratios greater than 1.0 can also be observed if lithium salts or hypertonic saline and urea are administered instead of the potassium salt, suggesting that cellular dehydration is a stimulus to K secretion. In water diuresis, only small amounts of potassium will be found in the urine, and injection of a mercurial diuretic may produce increased potassium excretion, suggesting diminished reabsorption of potassium.

The postulation of a reabsorptive mechanism and an active potassium-secreting tubular function can explain these findings. They also imply that mercurial diuretics decrease potassium reabsorption and specifically inhibit

potassium secretion. One might speculate that in the proximal tubules active anion reabsorption occurs, accompanied by passive cation reabsorption to maintain anionic homeostasis and electrolytic neutrality, respectively. Then, cation, or at least potassium, secretion occurs in the more distal parts of the tubules, probably by an exchange mechanism, to establish cationic homeostasis.

K. G. B.

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BOOK REVIEWS

THE CARE AND BREEDING OF LABORATORY ANIMALS. Edited by Edmond J. Farris. New York, John Wiley & Sons, Inc., 1950. xvi + 515 pp. $8.00.

As biological research is increasingly concerned with the description of the reactions and adjustments of individuals to the more subtle environmental changes and chronic rather than acute states, there is a growing demand for healthy animals of known lineage—and the necessity for their maintenance under experimental circumstances in normal condition; a demand that arises at a time when a large fraction of those who present themselves for training in research are quite unfamiliar with the habits of animals, many of urban origin have never had a pet. Hence there is an increasing need for adequate source books dealing with the care and breeding of the more commonly used laboratory animals, a need which this excellent monograph by Dr. Edmond J. Farris and collaborators—fifteen in all—will do a great deal to satisfy. The book consists of a series of essays, some of which are miniature monographs in themselves, each prepared by an investigator who is a recognized authority on the care and breeding of the specific mammal or class of animals about which he writes.

Three of the essays are by well-known authorities in the New Haven area. Dr. G. van Wagenen has drawn on her broad experience with the colony in the Department of Obstetrics and Gynecology to prepare the chapter on the care and breeding of monkeys, a chapter that is as pleasant to read as it is informative and one that might serve as a model for anyone who is called upon to write along similar lines in the future. In addition to providing general information of value to anyone keeping monkeys, Dr. van Wagenen has added a host of important details, where to obtain the animals, feeders, clippers, and the like; items of information that are invaluable to the beginner. Dr. Lionell E. Strong and Dr. Leon F. Whitney are, respectively, the authors of the excellent chapters on the care and breeding of mice and dogs; both of these chapters convey to the reader the authors' keen interest in the animals they raise—and this is true of all the contributors—quite apart from the ultimate scientific investigation in which the animals are employed; each contributor has written with a touch of the enthusiasm of an animal fancier. Yet, the programs of care and maintenance outlined are those that can be approached with modest means and facilities.

The scope of the book is broad, treating in individual chapters each of the more common laboratory mammals including the hamster and the ferret, together with chapters on birds, reptiles, amphibia, and fishes. Strangely, but perhaps not inappropriately, there is a chapter on insects, drosophila, followed by a valuable account of the control of the pests and parasites common to laboratory animals. Each author appears to have been given fairly full rein with regard the preparation and as a consequence the book is, in some respects, unbalanced—about 100 pages are devoted to fishes, in contrast to 14 devoted to amphia and 35 on the rat. Similarly, there is a wide variation in the number of references that accompany each chapter. Accordingly, the reader is the more grateful to the authors who have written selectively rather than comprehensively and have limited their references to those pertinent to the subject.

This is an excellent book; one that should be available to all who expect to use animals for study as well as those individuals responsible for their care.

DONALD H. BARRON

THE TRANSMISSION OF NERVE IMPULSES AT NEUROEFFECTOR JUNCTIONS AND PERIPHERAL SYNAPSES. By Arturo Rosenblueth. Cambridge, Mass., The Technology Press and New York, John Wiley & Son, Inc., 1950. xiv + 325 pp. $6.

This book, dedicated to the memory of Dr. Walter B. Cannon, is an excellent review of the problems of chemical transmission in the nervous system.

The first section deals with chemical transmission at autonomic neuroeffector junctions. It omits some important topics, such as supersensitivity of denervated structures, because they have been treated by the author in another recent book. Emphasis is given to the transmission in the two systems—adrenergic and cholinergic—and problems concerning liberation, generalized effects, and mode of action of chemical mediators are discussed in different chapters. The first section deals also with the theory of excitatory and inhibitory sympathins, the distribution of autonomic nerves, and the properties of smooth muscles, and ends with theories of transmission in autonomic neuroeffector systems, accepting the fact that the release of chemical agents is the exclusive way for transmission.

Twelve years have passed since the appearance of the monograph *Autonomic Neuroeffector Systems* by Dr. Cannon and Dr. Rosenblueth, and in this period of time the theme which constitutes a brief chapter in the first book—transmission in peripheral synapses—has developed so much that it constitutes the entire second section of the present book. As a theory of the mechanism of peripheral synaptic transmission, it is accepted that acetylcholine is the essential transmitter; K ions would be an important adjuvant, and the spike potential has the limited but important action of determining the release of acetylcholine.

Dr. Rosenblueth presents theories without dogmatism, and he discusses them in an exciting manner. This technique of writing offers great flexibility and allows the reader to adopt a personal opinion about the theories of chemical transmission. Nevertheless, in some of the chapter, as in the account of the action of potassium ions, it would be interesting to have more criticism concerning the role of potassium referring to other papers, as for example, those by Lorente de Nó.

The style of the book permits easy reading, an especially hard task to accomplish in controversial problems. Summaries at the end of each chapter and also at the end of each section help to state the most important facts in the sometimes elaborate discussion of contradictory experiments. An excellent bibliography comprising 720 papers is included, and also a comprehensive subject and author index.

J. DELGADO

THE ENZYMES, Vol. I, Part I. Edited by James B. Sumner and Karl Myrbäck. New York, Academic Press, Inc., 1950. xvii + 724 pp. $13.50.

This volume is the first of four which will serve as a comprehensive study on enzymology. The entire work has involved the collective labor of approximately 75 distinguished investigators and will contain 78 chapters,

of which 19 are included in the volume under review. This volume contains an excellent introductory chapter by the editors and thorough discussions of the following topics:

Physical Chemistry and Chemical Kinetics of Enzymes (E. A. Moelwyn-Hughes)
Enzyme Specificity (B. Helferich)
Enzymes in Relation to Genes, Viruses, Hormones, Vitamins, and Chemotherapeutic Drug Action (M. G. Sevag, J. S. Gots, and E. Steers)
Cytochemical Foundations of Enzyme Chemistry (A. L. Dounce)
Modern Aspects of Enzymatic Adaptation (S. Spiegelman)
Enzyme Inhibition (L. Massart)
Enzymes and Immunology (J. R. Marrack)
Enzymes Hydrolyzing Fats and Esters (R. Ammon and M. Jaarma)
Acetylcholine Esterase and Choline Esterase (K. Augustinsson)
Phosphatases (J. Roche)
Metaphosphate and its Enzymatic Breakdown (B. Ingelman)
Sulfatases (C. Fromageot)
Invertase (C. Neuberg and I. Mandl)
alpha-4-Glucosidases (A. Gottschalk)
beta-Glucosidase (S. Veibel)
Hydrolysis of Galactosides, Mannosides and Thioglycosides (S. Veibel)
beta-Gluruconidase (W. H. Fishman)
Amylases and the Hydrolysis of Starch and Glycogen (K. Myrbäck and G. Neumuller)

The appearance of the succeeding parts of this encyclopedic treatise will provide enzyme chemists with a reference work of inestimable value. The editors and publishers are to be commended for their courage in embarking on this impressive enterprise.

JOSEPH S. FRUTON

METHODS IN MEDICAL RESEARCH, Vol. 3. Ralph W. Gerard, Editor-in-chief. Chicago, The Year Book Publishers, 1950. xii + 312 pp. $7.00.

This series of monographs on methods in medical research was instituted for the purpose of assembling and appraising some of the more generally accepted research techniques utilized in the solution of experimental problems in medicine, and to describe and explain certain modifications in these techniques which have been mentioned but not adequately discussed in print. This latest volume is divided into four self-contained sections: Genetics of Micro-organisms, Assay of Neuro-humors, Selected Psychomotor Measurement Methods, and Methods for Study of Peptide Structure. Distinguished workers in each of these fields of research have been invited to elaborate upon the separate disciplines involved. Individual articles within the sections frequently have critical comments appended by the section editor or another worker who exposes other aspects of methods already described or illustrates an entirely different methodological approach.

The section on genetics of micro-organisms is edited by S. E. Luria and covers the problem of investigating genetics in bacteria, yeast, and neurospora. Section II on neurohumors, edited by J. H. Gaddum, details experimental methods for the determination of acetylcholine, choline-esterase, adrenaline, and nor-adrenaline in nerves and other tissues. The authors of special articles include Kenneth Harrison, W. Feldberg, F. C. MacIntosh, W. L. M. Perry, Bruno Mendel, and Rosemary Hawkins, and U. S. von

Euler, in addition to Dr. Gaddum, and there are addended commentaries by John Welsh, Andrew Wilson, and Maurice Tainter. Altogether this is a very useful and stimulating section. Walter R. Miles of Yale edits the selected psychomotor measurement methods and includes in this, the longest section in the book, methods for measuring physical work and strength, reaction time, coordinated motor response, manual dexterity, eye movement coordination, and motor tests of laboratory animals. Included in the list of authors for this section are Josef Brožek, Neal Miller, Walter Miles, L. P. Herrington, R. B. Ammons, H. E. King, and J. Clausen, and the Crawfords, among others. The fourth section concerns methods for study of peptide structure and is edited by Choh Hao Li. Here are to be found articles by J. C. Lewis, R. R. Porter, Henry Bull, Joseph Fruton, and Emil Smith.

Most of the articles are directly practical and brief. The over-all editing performance is of good quality and the book is well indexed. References given in some of the sections unfortunately do not include titles of the articles, but each section has a fairly representative list of original sources. Naturally enough, the book suffers from the fact that methods covered within each section had to be limited to those thought to be most useful or most generally acceptable. The chief virture of *Methods in Medical Research* lies in its direction of attention toward the value of critical analysis of methods. One can even imagine the influence of this series of monographs giving rise to a more widespread practice of teaching the basic methods of research. This would in itself constitute a fundamental contribution to scientific development.

ROBERT B. LIVINGSTON

ESSENTIALS OF MEDICINE, 16th ed. By Charles Phillip Emerson and Jane E. Taylor. Philadelphia, J. B. Lippincott Co., 1950. 815 pp. $4.00.

This edition of the well-known medical nursing text has been thoroughly revised and manifestly improved. There is a new and more readable format presenting the generally excellent material which has been carefully rewritten to include work with the newer drugs, treatments, and preventive medicine. Several early chapters have been added in an attempt to point the nursing care toward the treatment of the patient as a person. There is a brief prospectus, so-called orientation, setting the stage for the separate sections. Even the arrangement of the chapters has been improved, and a current list of references follows each subject. An intriguing clinical situation is presented with thought-provoking questions at the end of each unit, a device which should easily succeed in interesting the student. Above all, the entire text is keyed to the nursing situation as related to the individual patient. Moreover, the nursing care discussion has now been made an integral part of each section rather than as an appendix as in the previous edition.

The end result is a most usable text, and it is to be hoped that future revisions will follow frequently.

ELIZABETH BARBER

THE FROG. ITS REPRODUCTION AND DEVELOPMENT. By Roberts Rugh. Philadelphia, The Blakiston Company, 1951. x + 336 pp. $4.25.

The need for a text in amphibian embryology embodying information obtained by descriptive and experimental methods has been apparent for

some time. The compilation of a book of this type requires that the author have considerable experience in teaching and close contact with current literature. Since Rugh has been active in research and teaching of experimental embryology for some years, it was anticipated that he would produce a text that would be satisfactory to both student and instructor.

The author opens with an historical background of the study of embryology. There follow sections on the reproductive system of the adult, cleavage, blastulation, gastrulation, early organogeny, and the germ layer derivatives. The general organization and physical makeup of the book are excellent. The illustrations are perhaps the finest to appear in a text on this general subject, although the convention of numbering illustrations was ignored in spite of its pedagogical usefulness.

No account of early amphibian embryology can be considered complete without reference to data obtained by experimental methods. It is in the presentation and interpretation of the experimental work of others that the author is least effective. It is disappointing to find perpetuated without discussion certain concepts of the mechanisms and processes of embryonic development which have been rendered doubtful or refuted by investigations of none-to-recent date. Thus the roof and walls of the archenteron are represented as originating by delamination from material which has passed over the dorsal lip of the blastopore without reference to the conflicting experimental evidence obtained by Vogt twenty-two years ago. Personal opinion, information backed by experimental evidence, and hypothesis are often presented without identification. The result is confusing.

Subsequent sections dealing with later descriptive embryology are considerably improved, being well organized and illustrated. However, this description, like the rest of the book, suffers from lack of documentation, thus denying the interested student rapid access to the literature on a particular subject. In general, the text frequently appears to have been written in haste and reread in the same manner. There are pronouns with doubtful antecedents, sequences of events are described in such a way as to leave the order in doubt, and not infrequently the construction of sentences and paragraphs is so awkward that many rereadings are necessary before one can grasp what the author is trying to express. An example may be found on page 170 where four consecutive sentences begin with the word "this." On the whole, it may be said that in spite of the excellence of the illustrations this is not the careful, scholarly work that the student of embryology deserves and demands.

R. R., JR.

CURRENT THERAPY 1951. Edited by Howard F. Conn. Philadelphia, W. B. Saunders Company, 1951. xxxi + 699 pp. $10.00.

Current Therapy is an annually revised volume designed to present, for all diseases, the methods of therapy actually in present use by leading authorities, the discussion of these methods being written by the authorities themselves. Therapy alone is discussed, presumably in order to keep the size of the volume within reasonable bounds and to make frequent revision of the entire volume practicable. Diseases are arranged into fifteen sections, largely according to the anatomical systems, and an excellent index is provided, making the volume suitable for ready reference by practitioners. Thus the most acceptable modern methods of therapy are presented, concisely yet thoroughly enough for practical use, and arranged so as to be

rapidly accessible. The 1951 edition of *Current Therapy* continues to serve excellently this rather important function.

R. G.

NUTRITION IN HEALTH AND DISEASE, 11th ed. By L. F. Cooper, E. M. Barber, H. S. Mitchell, and H. J. Rynberger. Philadelphia, J. B. Lippincott Co., 1950. xvii + 744 pp. $4.00.

The fact that there are so many editions of this textbook, which first appeared in 1928, gives one a clue to the importance which the authors attach to keeping nutrition books up to date and why their book remains a favorite among teachers of nutrition and diet therapy.

Information is presented in a clear and very readable manner. Many pictures, graphs, and diagrams make for added interest. The charts and tables throughout the book are well chosen and give pertinent facts.

The long association of the authors with medical institutions both as dietitians and as teachers of nurses and dietitians has provided them with a good philosophy of nutrition and the ability to interpret nutrition in its many ramifications to students of the subject. Although the book is designed primarily for student nurses and dietitians, all persons interested or connected in any way with the field of nutrition will find the book an excellent reference.

For writing a book of value to students with varying backgrounds and experience, the authors deserve much credit. There are few textbooks which compare with this one in scope and yet give sufficient detailed information. In the section dealing with Diet in Disease, the reader is never allowed to forget that all dietary modifications are based on the normal diet and that in all therapeutic diets one must consider the total nutrition of the patient.

The authors have also recognized the role of economics in the feeding of individuals and of families. They have included ample information on low cost meal planning as well as inexpensive family-size recipes. The inclusion of such information is only one more reason for crediting the authors with a major contribution to the study of nutrition.

MARGUERITE J. SCHAEFER

EXPERIMENTAL DESIGNS. By William G. Cochran and Gertrude M. Cox. New York, John Wiley and Sons, Inc., 1950. 454 pp. $5.75.

All experiments require a decision as to how to organize the available units of experimental material—a decision perhaps made only after long and tortured deliberation, or perhaps with an absolute innocence of conscious thought. The results of an experiment always reflect this arbitrary plan or form. Results can be described as answers obtained to a set of questions—quantitative estimates or just yes or no—together with evidence for the reliability of the answers—again, standard deviations or simply the general appearance of plausibility. The adequacy of the plan determines whether the estimates really apply to the questions or are biased by the measurement of extraneous variables at the same time. Also critical is the effect of the plan on the reliability of the estimates. We can define the field of experimental design as the study of the effect of the form of an experiment on its results.

The book, *Experimental Designs,* presents a catalogue of available designs, with details of computation and discussions of the most suitable

applications of each. The computations are given with all possible directness and economy, giving readers the benefit of great care and comprehensive experience. The procedures shown include estimation of treatment effects and the isolation of component treatments in factorial experiments, testing of significance by t-tests and analysis of variance, removal of extraneous variation from the treatment calculations, estimation of confidence limits for treatment effects, and the use of missing value formulae for the repair of damaged data. All new methods are illustrated by worked examples. The discussion of uses and limitations of each type of design, and the general observations on method in the introductory chapters are clear and suggestive. For help in estimating the advantages of a split-plot design, to find what incomplete block plans are available to fit an experimental problem, to practice the analysis of lattice squares, or for suggestions in combining the results of several experiments, this is without any question an authoritative handbook.

Experimental Designs is particularly useful in situations where experimental material is available but expensive, and where the maximum precision obtainable per unit of material is worth obtaining. It is easy to think of experiments of this type. It is also easy to recall others where material and observations are cheap and where answers are required as quickly as possible, or again where the material is finite so that the results must be as suggestive as possible on many questions, without approaching certainty on any one. In the first situation "inefficient" methods are often available which sacrifice material in favor of speed or convenience. Also the use of sequential procedures, where an experiment can be stopped as soon as a given precision is reached, may save considerable time as well as material. With severe limitations on material and many questions to be answered, the use of fractional replication (see p. 189) may be worth considering; intuition may also be fruitful.

Experimental Designs does not undertake such a general treatment of design from the experimenter's point of view. (This and the description of computations could not have been done at the same time.) Efficient, and many elaborately planned, experiments are emphasized, at the expense of others perhaps equally frequent, by virtue of their having designs that fit the pattern of the book.

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